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Synthesis, Characterization and Anticancer Activity of Chitosan Schiff Base / PEG Blend Doped with Gold and Silver Nanoparticles in Treatment of Breast Cancer Cell Line MCF-7

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Abstract

In the present study, chitosan Schiff base has been prepared from chitosan reaction with p-chloro benzaldehyde. The AuNPs and AgNPs were manufactured by extract of onion peels as a reducing agent. The AuNPs and AgNPs that have been synthesized were characterized through UV-vis spectroscopy, XRD analyses and SEM microscopy. The polymer blends of the chitosan / PEG has been prepared by using the approach of solution casting. Chitosan Schiff base / PEG Au and Ag nanocomposites were synthesized, nanocomposites and polymer blends have been characterized by FTIR which confirm the formation of Schiff base by revealing a new band of absorption at 1693 cm-1 as a result of the (C=N) imine group. FESEM, DSC and TGA confirm the thermal stability of the prepared polymer blends and nanocomposites, nano composites have shown good results in inhibiting breast cancer cell line MCF-7, IC50 of nanocomposite = 19.44 µg / mL.

Keywords: Anticancer cell line, Chitosan, Schiff base, PEG, Nano composite.

تحضير، تشخيص والفعالية المضادة للسرطان لقاعدة شيف للكيتوسان/ بولي اثلين كلايكول والمتضمن لدقائق الذهب والفضة النانوية في معالجة الخط السرطاني للثدي.

مها عبدالوهاب يونس*[١]، بكر فوزي عبدالله[٢] ، نبراس يوسف عبدالله[٣] و ابراهيم جليل ابراهيم[٢]

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الخلاصه

في الدراسة الحالية تم تحضير قاعدة شيف للكيتوسان من تفاعل الكيتوسان مع بارا كلورو بنزالديهايد. تم تحضير دقائق الذهب والفضة النانوية بواسطة مستخلصات قشور البصل كعامل مختزل.شخصت دقائق الذهب والفضة النانوية المحضرة بواسطة مطيافية الاشعة فوق البنفسجية-المرئية , طيف حيود الاشعة السينية والمجهر الالكتروني الماسح. تم تحضير خلائط قاعدة شيف للكيتوسان /بولي اثلين كلايكول بطريقة خلط المحاليل. تم تحضير المتراكبات النانوية مع الذهب والفضة للخلائط المحضرة. شخصت الخلائط المحضرة والمتراكبات النانوية بواسطة مطيافية الاشعة تحت الحمراء والتي اكدت تكوين قاعدة شيف بظهور قمة مجموعة الإمين عند 1693cm- وبواسطة المجهر الالكتروني الماسح والتحاليل الحرارية التي اثبتت الاستقرارية الحرارية للخلائط والمتراكبات المحضرة.اظهر المتراكب النانوي فعالية جيدة في تثبيط نمو خلايا الثدي السرطانية-MCF 7 IC50 للمتراكب النانوي = 19.44 µg / mL .

الكلمات المفتاحية: الخط المضاد للسرطان, الكيتوسان, قاعدة شيف, بولي اثلين كلايكول, المتراكب النانوي.

Introduction

Biopolymers are synthetic polymers produced from biological materials, either chemically or entirely biologically, by living organisms. Yet biopolymers generally have weak mechanical characteristics, limited processing options, and limited end-use applications. In order to get over these restrictions and produce complex materials for a range of applications, biopolymers can be reinforced with fillers or nanofillers [(1)]. Chitosan is a linear polysaccharide made of D-glucosamine and N-acetyl-D-glucosamine units, because of the functional groups that it carries, chitosan has excellent adsorption properties for other substances.

It has primary, the secondary hydroxyl (OH) groups on the C-3 and C-6 positions as well as an amine/acetamide group on the C-2 [(2)]. It has several uses in the food, chemical, drug delivery,

drug release and drug carrier industries. Moreover, it is non-toxic and biodegradable [3]. Aldehydes, ketones, and primary aliphatic, aromatic and heteroaromatic amines frequently form Schiff bases when they react with carbonyl substances (aldehydes and ketones). An azomethine (-C=N-) bond can be found in Schiff bases. It is generally known that Schiff bases have characteristics that are antimicrobial, antiviral, anticancer, and antifungal. In addition to being used as acid catalysts, reduction catalysts, and oxidation catalysts. Schiff bases also have a unique affinity for metal ions. Schiff bases have great bioactivity due to their capacity for intermolecular hydrogen bonding and proton transfer equilibrium [4]. The polymer blend is an intriguing technique for creating novel materials for particular purposes since it is quick, easy, and inexpensive. One can create a new substance with the combined qualities of its components by mixing them. It is an economical technique for creating novel materials with desired properties [5]. Polyethylene has been extensively utilized to modify the surfaces of medical devices, nanoparticles, pharmaceuticals, liposomes and biomolecules like peptides, proteins, enzymes and nucleotides [6]. An expanding number of goods and applications are using nanomaterials. The rapid development of nanotechnology necessitates safety evaluations of its effects on both human health and the environment. Even though the fundamental material is the same, materials at the nanoscale might behave differently from those at greater scales. Different chemical, physical, electrical, and biological characteristics may be found in nanoscale materials [7]. One of the materials now being investigated the most is gold nanoparticles, which are employed in numerous fields, including optoelectronics and catalysis [8]. In the modern world, silver nanoparticles are one of the most researched and promising prospects for novel and effective uses, with impressive findings being reported in the pharmaceutical sciences [9]. Multi-phase materials called nanocomposites have at least one phase with a size in the nano range (10 –100 nm). Nanocomposite materials have recently come to light as viable options to alleviate the shortcomings of many engineering materials. These are reportedly the 21st century's materials. Dispersed matrix and dispersed phase materials are two categories of nanocomposite materials [10]. Breast cancer is a disease that is highly heterogeneous, encompassing a group of genetically and epigenetically distinct diseases with diverse clinical features, and it could serve as an infinite source of homogenous self-replicating materials using simple yet standard media and approaches. A sizable portion of current knowledge on breast carcinomas is derived from in vivo and in vitro studies using breast cancer cell lines [11].

Materials and Methods

Preparation of the Chitosan Schiff Base (Cs)

Chitosan 0.5 g has been dissolved into 2% of the acetic acid and heated for 1hour at 60 °C, while 4-chloro benzaldehyde 1g was dissolved in 20 mL of ethanol. This mix has been magnetically stirred and heated at a temperature of 60 °C with reflex for 12 hours. The crude product was washed with ethanol after cooling and then dried in the oven at a temperature of 50 °C for 24 hours. [12] Chitosan Schiff base's synthetic route is depicted in Figure1.

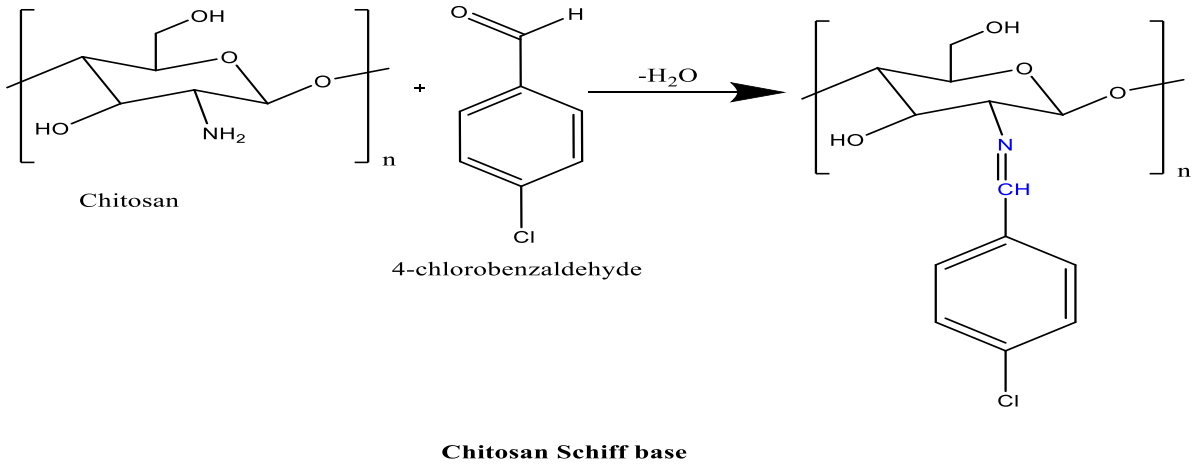


Figure 1. Synthesis of the Chitosan Schiff base

Polymer blend preparation

The chitosan Schiff base was prepared by dissolving 1g of the chitosan Schiff base into 100 mL of 2% percent aqueous acetic acid solution and

stirring for 1 hour at a temperature of 60 °C, 5 g PEG was dissolved into 100 mL of water in order to prepare 5% percent W / V polymer solutions, 10 mL of Cs and 5 mL PEG polymer solutions were mixed in order to prepare a homogenous solution by the using a hot-plate stirrer for 30 min. Cs / PEG mixes were made by combining different Cs: PEG ratios (10:5) [13].

Preparation of crude extract (Onion Peels)

To produce onion peel extract, 10 g of onion peels were dissolved in 100 mL of deionized water. For the purpose of obtaining onion peel extract at 200 ppm, 0.02 g of the produced powder was dissolved in 100 mL of deionized water. The fresh extract was taken from the onion peels and used as a reducing and stabilizing agent. This mixture was stirred, heated at 50 °C for 2 hours, and the resulting substances were filtered before being dried in an oven at 50 °C [14].

Preparation of $HAuCl_4$.$3H_2O$, $AgNO_3$ solutions

To produce the stock solution, 1 g of gold chloride trihydrate ($HAuCl_4.3H_2O$) was dissolved in 100 mL of deionized water. Next, 2 mL of the stock solution was taken and diluted to 100 mL using consecutive dilution methods (100 ppm). In order to create $AgNO_3$, (0.016g) of AgNO3 was dissolved in 100 ml of deionized water (100 ppm). Green nanoparticle synthesis of Au and Ag Three ml of aqueous onion peel extract was added to ten ml each of silver nitrate and aqueous gold chloride solution. The final mixture was stirred for ten minutes at 25 °C [15]. Indicating the formation of AuNPs, the color of gold changed from yellow to purple. Indicating the formation of AgNPs, the color of silver changed from colorless to brown. The precipitate is taken, collected, and diluted with deionized water after being separated from the filtrate by a centrifuge spinning at 10,000 revolutions per minute [16].

Preparation of Cs / PEG gold and silver nanocomposites

To produce nanocomposites, mixtures of 10 mL of chitosan Schiff base, 5 mL of PEG, 12.5 mL of AuNPs and 12.5 ml of AgNPs in two different concentrations (75, 150 ppm) were combined. After stirring for two hours, the mixture was cast onto Petri dishes and kept in a constant temperature oven at 50 °C for 24 hours [17].

Cell cultures

MCF -7 cells have been cultured in RPMI 1640 with 10% fetal bovine serum, 100 g/mL streptomycin, and 100 units/mL penicillin. The cells have been passaged twice each week with Trypsin-EDTA, then reseeded at a confluence of 80% and incubated at 37 °C [18,19].

Cytotoxicity assays

MTT test was utilized in 96-well plates in order to investigate the cytotoxic impacts of nanocomposite [20, 21]. 1×10^4 cells per well were planted in each cell line. Cells were treated with nanocomposite after 24 hours after a confluent monolayer had been established. After 48 hours of the treatment, cell viability has been determined through removing medium, adding 28 mL of a 2mg/mL MTT solution, and incubating the cells for 2.5 hours at 37 °C. After removing the solution of the MTT, crystals within wells were solubilized through the addition of 130µL of DMSO (Di-methyl Sulphoxide) and incubation at 37 °C for 15 minutes with shaking [22]. Absorbency was evaluated at 492 nm with the use of micro-plate reader and the test was done in triplicate. The equation below was utilized in order to compute the cell growth inhibition rate (cytotoxicity percentage) [23, 24].

Rate of Inhibition =

(A- B) / A ×100 ---------- (2-1)

where A is the control optical density and B is the sample optical density [25]. Cells were seeded into 24-well micro-titration plates at a 1×10^5 cells mL^{-1} density and cultured for 24 hours at 37 °C in order to examine them under inverted microscope. After that, cells were exposed to the nanocomposite for a period of 24 hours. Plates were stained with crystal violet dye and then incubated at a temperature of 37 °C for 10 –15 minutes after exposure duration [26]. The stain was carefully wiped away with tap water to an unstained point. Cells were seen under a 100× magnification power using inverted microscope and images were taken using a digital camera that had been mounted to the microscope [27, 28].

Results and Discussion

Characterization of AuNPs and AgNPs

Measurement of UV-visiblespectroscopy

The solution containing AuNPs and AgNPs has a UV-Vis spectrum, which is shown in Fig. 3. UV-Vis spectroscopy is a technique that may help determine whether metal nanoparticles were produced in an aqueous solution. A wide (SPR) band appeared at 540 nm in Figure. 2 The UV-Vis spectra of an AuNPs solution, indicating the synthesis of AuNPs. A large surface plasmon resonance band appeared at 425 nm in Figure.2B of the UV-Vis spectrum of an AgNPs solution, confirming the formation of AgNPs [29]. The SPR of AgNPs and AuNPs was thought to be responsible for the interaction between free electrons on the metal surface and incoming light [30].

X-ray Diffraction Analysis (XRD)

The mixed (AgNPs and AuNPs) that had been synthesized were evaluated for their crystallinity using X-ray diffraction (XRD) analysis. Table 1 and (Figure. 3) display the XRD results of the generated AuNPs and AgNPs. The 2theta degree of dried AuNPs and AgNPs ranged from 10 to 90. According to AuNPs showed sharp, strong peaks at 38° and 77° nm, which mapped to the Braggs planes (111) and (311), respectively (JCPDS 04-0784) [32]. Showing the face-centered cubic structure of the generated AuNPs. In the case of AgNPs, the peak at

2theta values of 32°, 44°, and 64° corresponds to Braggs reflections (111), (200), and (220), respectively (JCPDS 04-0783) [33].

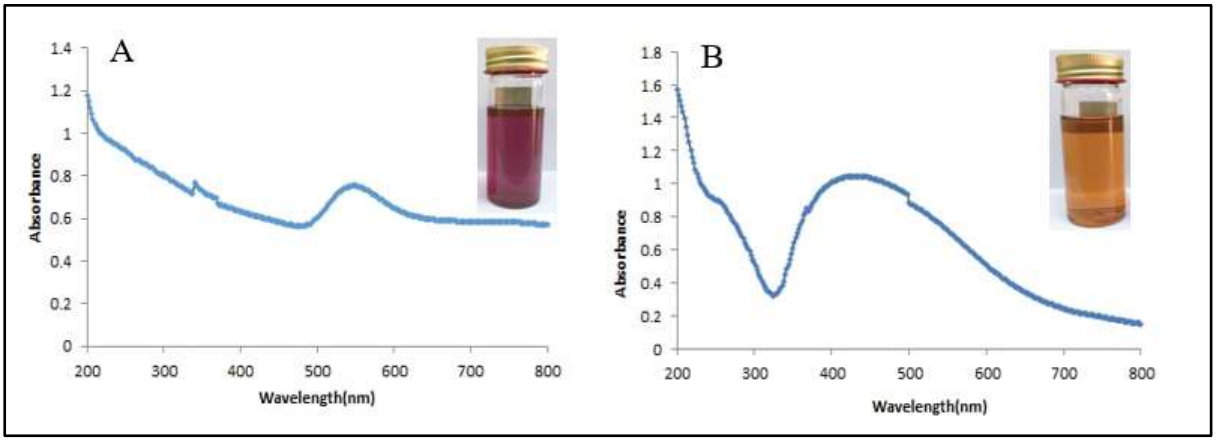


Figure. 2.UV-Vis spectrum of A-AuNPs and B- AgNPs [31].

Table 1.XRD examination of AuNPs and AgNPs yielded calculated crystallite sizes for all allocated and D average peaks

Element	2 theta	FWHM	Hkl	D	D average
Au:Ag	32.3	0.492	111	3.36 nm	5.63 nm
	38.3	0.393	111	4.61 nm	
	44.7	0.885	200	2.22 nm	
	64.8	0.787	220	4.18 nm	
	77.8	492	311	13.8 nm	

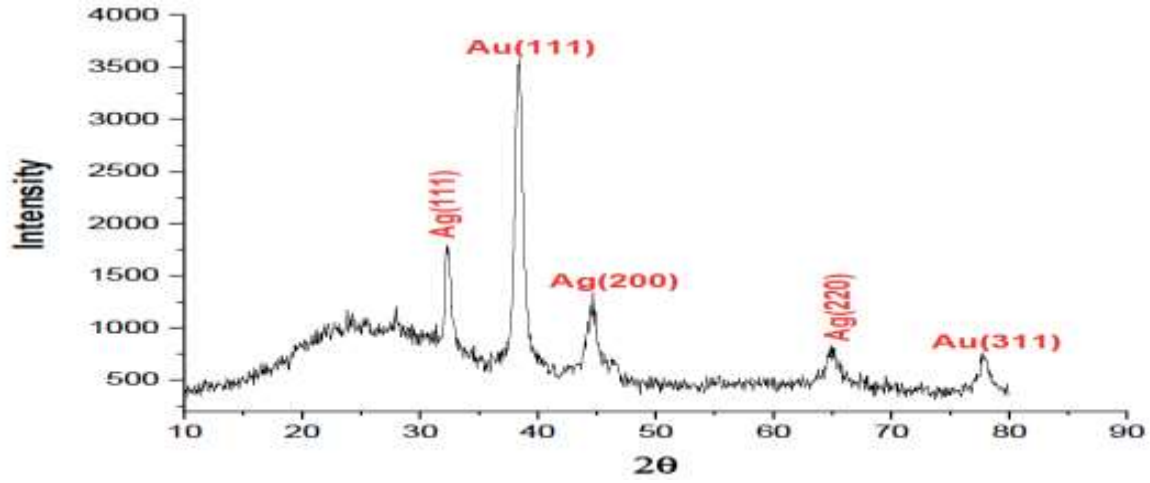


Figure 3. XRD Patterns of AuNPs and AgNPs

Characterization of chitosan Schiff Base, blend and nano-composites

FTIR Analyses

Chitosan Schiff base Figure.4 revealed a new band of absorption at 1693 cm^{-1} as a result of the (C=N) imine group, an absorption band at 1525 cm^{-1} as a result of C=C of 2- Chloro O–C) pairing stretching vibration in (1-4) glycosidic bonds and additional peak values at 1076.28 cm^{-1} [34].

Filed Emission Scanning Electron Microscope (FESEM)

The surface morphology, crystallinity, size, and phase locations of the produced material could all be researched by the examinations of FESEM [35]. Surface morphology varies for chitosan Schiff base, PEG, Au and Ag nanoparticles, polymer blends, and nanocomposites were investigated with the use of the FESEM approach. The FESEM images for pure gold and silver nanoparticles are shown in Figure.5. D, the average nanosize of the particles is ranged between 30 - 40 nm for gold and silver nanoparticles. The FESEM images revealed that there were significant changes on the surface of prepared blends after interaction between the polymers. Nanoparticles in a homogeneous distribution over the matrix's surface. The particles in the nanocomposite film were found to have an almost spherical morphology. However, some nanoparticles agglomerations were found on the rough surface of the nanocomposite.

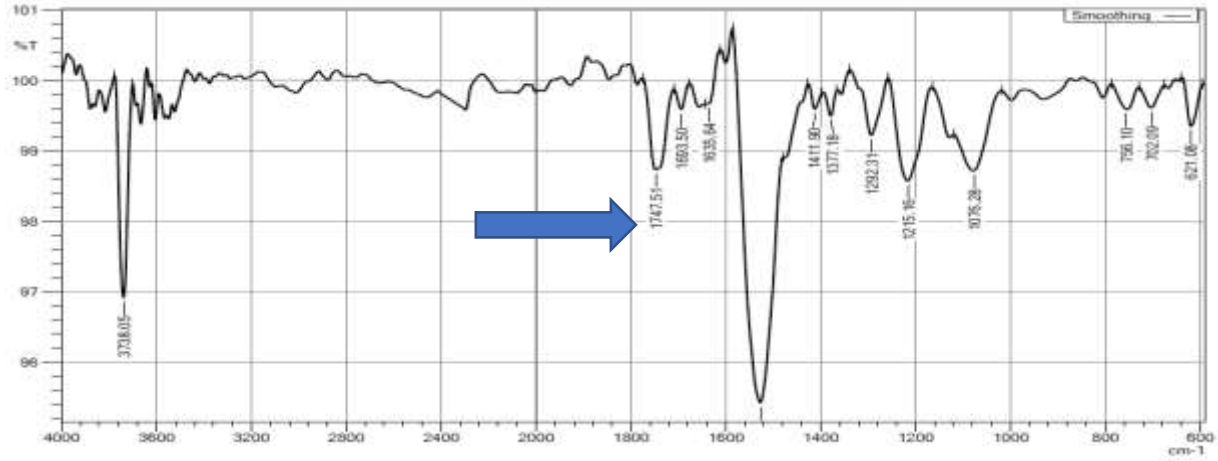


Figure. 4. FTIR Spectrum of chitosan Schiff base

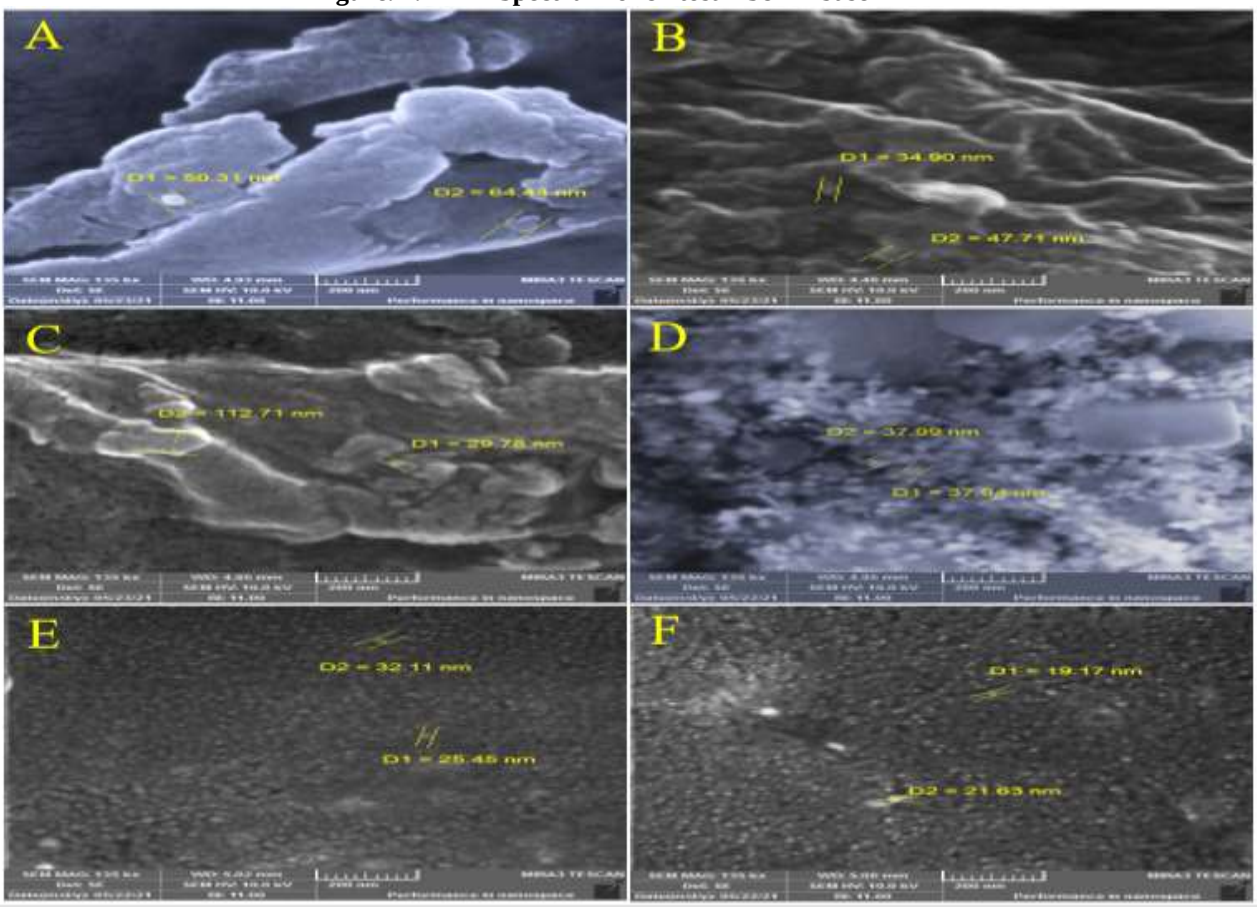


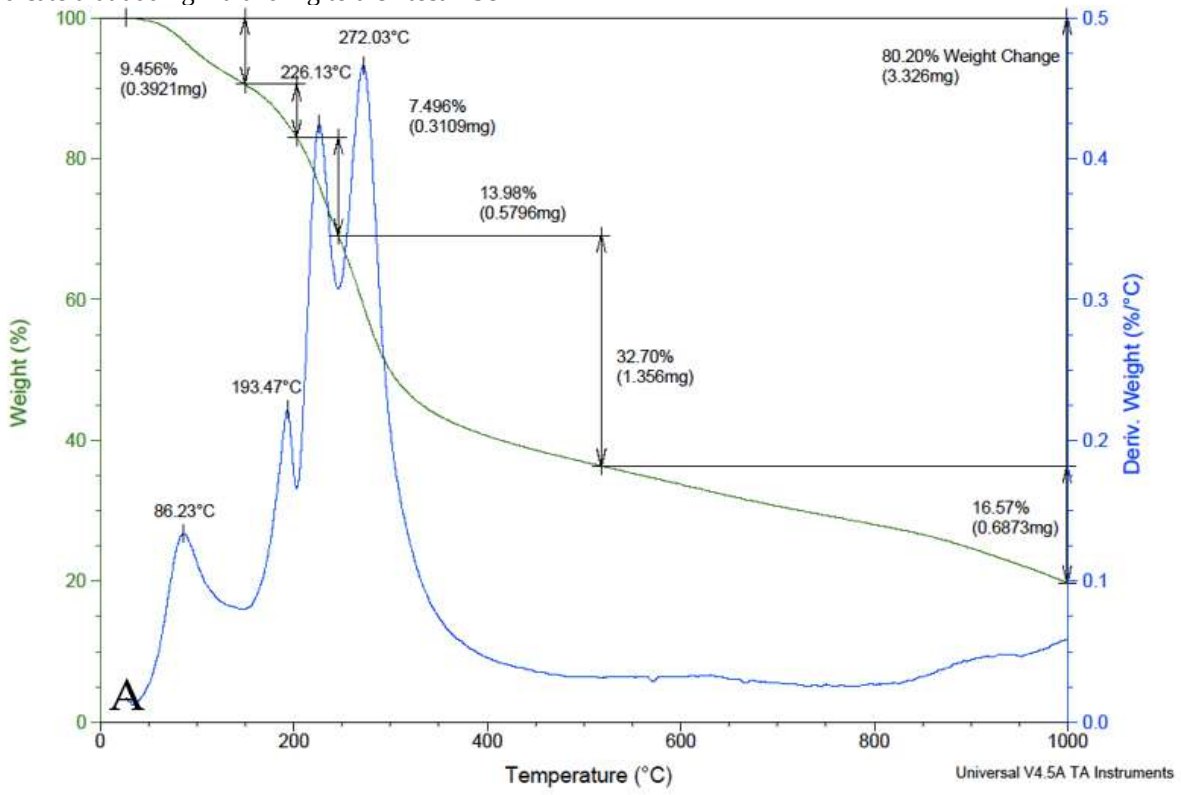
Figure 5. FESEM image of A- chitosan Schiff bases, B- PEG, C- Blend (Cs, PEG), D- gold and silver nanoparticles E- Nano-composite (Cs, PEG and AuNPs, AgNPs 75 PPM), F - Nano composite (Cs, PEG and AuNPs, AgNPs 150 PPM).

Thermal analysis

The thermal gravimetric (**TGA**, **DSC**) analysis of chitosan Schiff base, Cs Schiff base / PEG polymer blend ,and chitosan Schiff base / PEG Au and Ag nanoparticles was carried out at temperatures ranging from 25 °C to 1000 °C at constant rate of 10 °C minutes. The **TGA** curve of the chitosan Schiff base Figure.6. A illustrates five sequence mass loss stages. The curve of **DSC** in Figure.6. A for the chitosan Schiff base showed a Tg of (86.23 °C). The peak at (272.03) °C concerning polymer melting Tm. The curve of the **DSC** of polymer blend in Figure.6. B shows Peak at the temperature of 398.75 °C concerning polymer melting Tm. The curve of the **TGA** of the chitosan Schiff base / PEG and Au, Ag nanoparticles Figure.6 C, illustrates 3 sequence mass loss stages. The curve of the **DSC** in Figure.6 C for the chitosan Schiff base / PEG and Au, Ag nanoparticles shows the peak regarding the crystalline temperature point Tc at (235.92 °C). Peak value at a temperature of (398.62 °C) concerning polymer melting Tm. [36, 37]. All temperatures were pushed a little higher. This higher thermal stability results from an aldehyde derivative that was supported by NPs. This results in a reduced rate of thermal breakdown, demonstrating the effect of gold and silver coordination bonding on thermal stability; also, the mixed film only shows one Tg on its thermogram. This indicates that the blend has hydrogen bonding connections between the chitosan Schiff base and PEG and that the two polymers are effectively mixed. These results indicate that adding Au and Ag to a chitosan Schiff base / PEG nanocomposite at such a low concentration can increase its thermal stability.

Anti-cancer cell line

The Anti-proliferative activity of nanocomposite has been tested by investigating its capability for inhibiting MCF-7 cell line proliferation. The results demonstrated the ability of prepared nanocomposite to destroy and kill of cancer cells as shown in Figure.7. NPs accumulate and entrap cancer cells to be able to kill them. The process can also be defined by the retention and penetration effect that abnormal lymphatic flow and angiogenic vessels impose on cancerous cells. As a result, in contrast to normal cells, the entration of these NPs accumulates more or more specifically inside cancerous cells. In case of gold nanoparticles, conjugation of these bacteria at the Au surface via cell surface receptors, such as antibodies, peptides, and antibiotics against tumor-infected cells, might increase the existence of these nanoparticles and enable their use in diagnosis and therapy (38). Nano silver's small size and spherical form induce a high rate of apoptosis. Additionally, apoptosis, oxidative stress, and loss of cell membrane integrity are all potential causes of cell damage brought on by silver nanoparticles[39]. The activity of nanocomposites against cancer cells is concentration - dependent manner. Inhibition rate nano composite at concentration 3.1, 6.25, 12.5, 25, 50 µg / mL equal 6.66, 25.33, 41.33, 59.33, and 83.33 respectively as shown in Figure.8. IC_{50} of nanocomposite = 19.44 µg / mL [40].



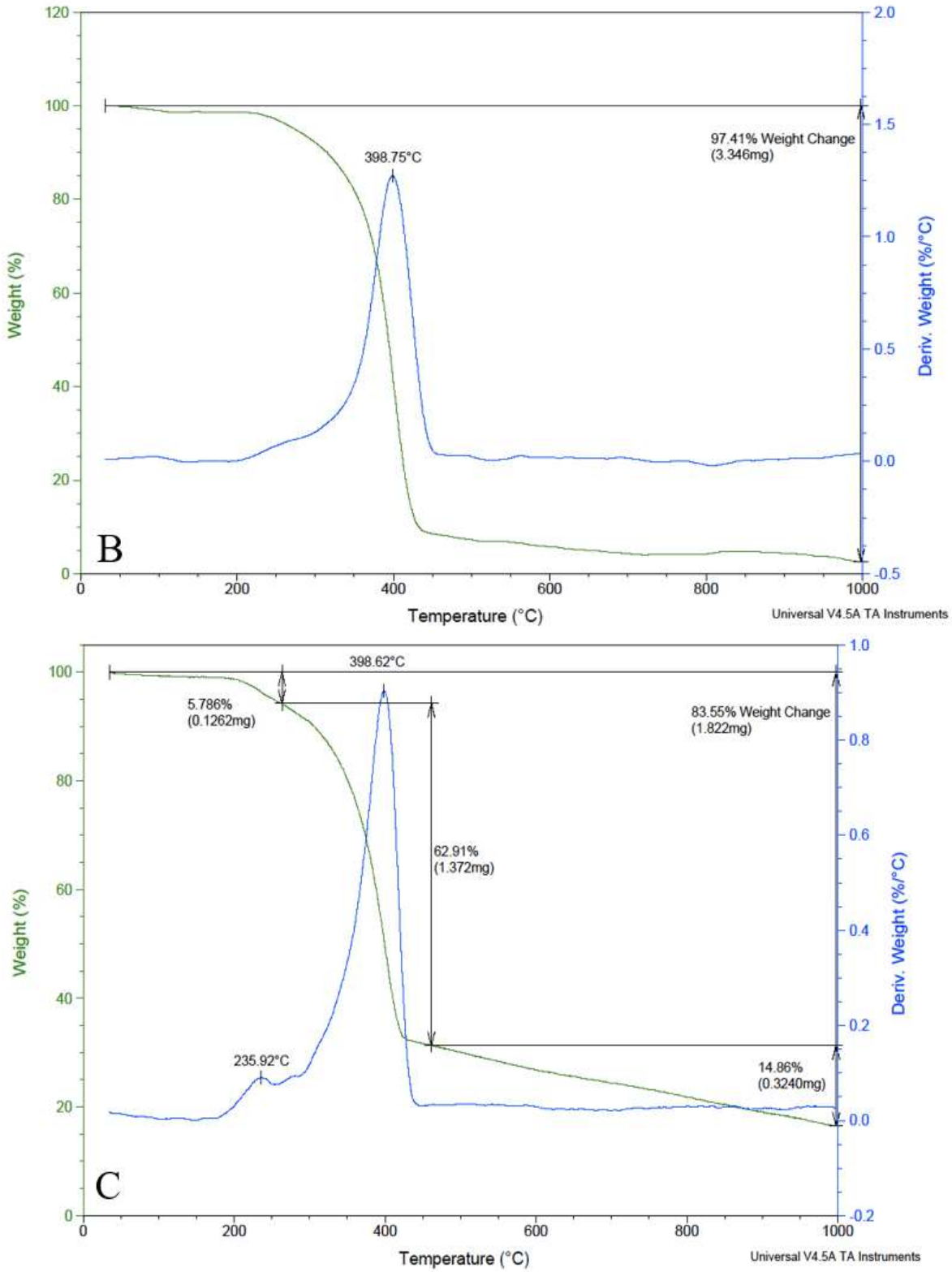


Figure 6. Thermal analyses (TGA and DSC) A- Chitosan Schiff base B- Blend (Cs and PEG), C- Nano-composite (Cs, PEG and Au, Ag nanoparticles).

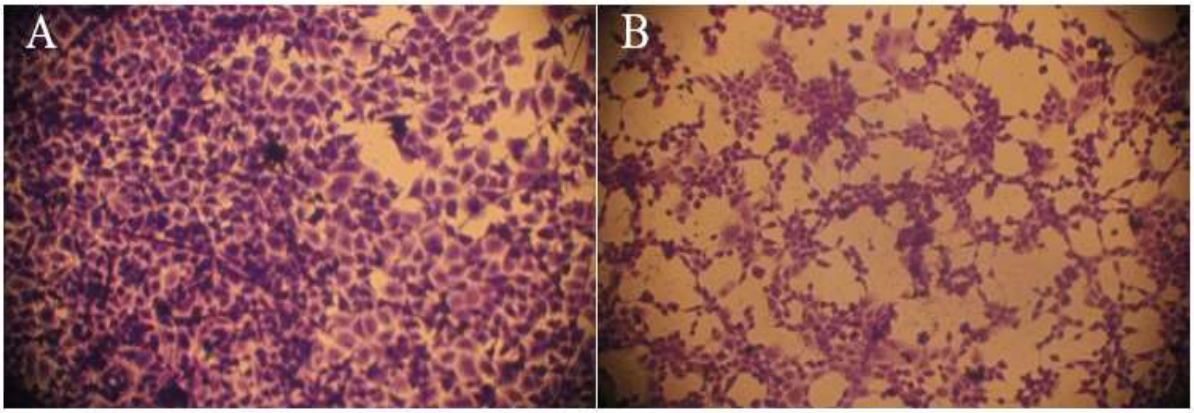


Figure 7. A- Control the untreated MCF-7 cells, B- Morphological changes in the MCF-7 cells after treated with nanocomposite.

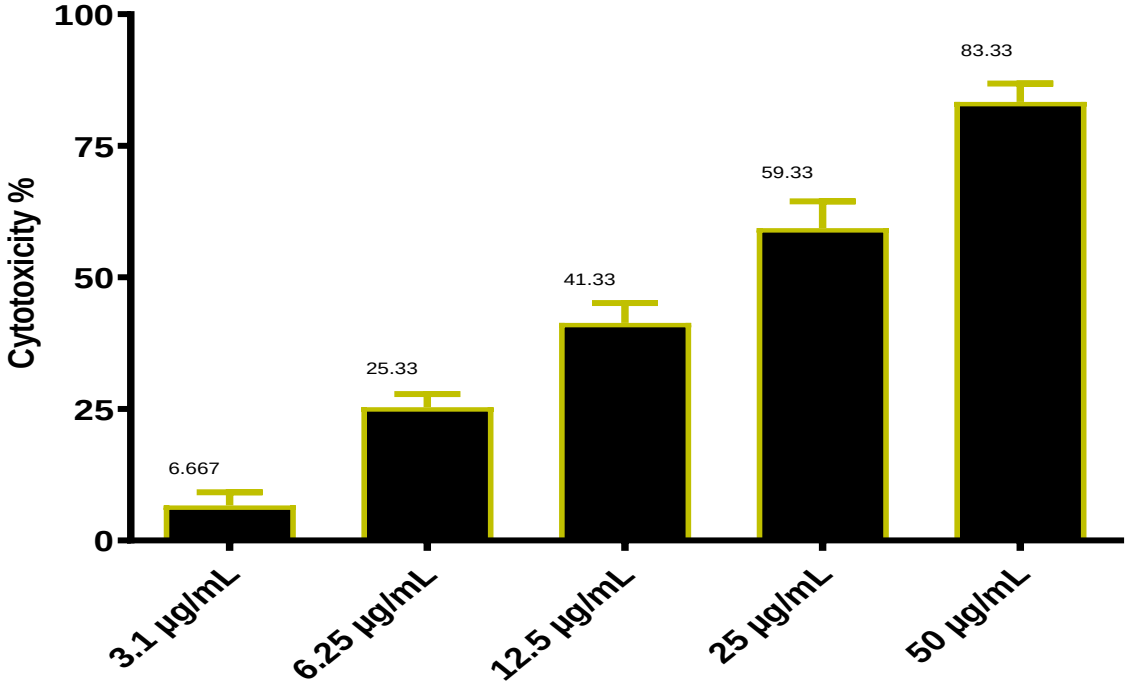


Figure 8. Percentage of Cytotoxicity of nanocomposite in MCF-7 cells.

Table 2.The significant differences (Tukey test) of Nanocomposite

The relationship between concentrations	N	Mean difference	√MSW/nh	HDS (q cal)	q crit	significant
3.1-6.25	3	3.15	2.092313977	1.50551018	4.37	NS
3.1-12.5	3	9.4	2.092313977	4.49263356	4.37	S
3.1-25	3	21.9	2.092313977	10.4668803	4.37	S
3.1-50	3	46.9	2.092313977	22.4153738	4.37	S
6.25-12.5	3	6.25	2.092313977	2.98712338	4.37	NS
6.25-25	3	18.75	2.092313977	8.96137014	4.37	S
6.25-50	3	43.75	2.092313977	20.9098637	4.37	S
12.5-25	3	12.5	2.092313977	5.97424676	4.37	S
12.5-50	3	37.5	2.092313977	17.9227403	4.37	S
25-50	3	25	2.092313977	11.9484935	4.37	S

Conclusions

The results of this study suggested that the addition of AuNPs & AgNPs has improved thermal stability of chitosan Schiff base / PEG based composite. Gold & Silver nanocomposites exhibited good anticancer activity in breast cancer cell lines MCF-7, IC_{50} of the nanocomposite = 19.44 µg / mL.

Conflict of Interest

There is no conflict of interest in the manuscript.

Funding

The work has no funding .

Ethics statements

The authors did not use animals in the study .

Author Contribution

The authors confirm contribution to the paper . all authors reviewed the results and approved the final version of the manuscript .

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