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Synthesis, Characterization and Anticancer Activity of Chitosan Schiff Base / PVP Gold Nano Composite in Treating Esophageal Cancer Cell Line

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Abstract:

In the present paper, chitosan Schiff base has been synthesized from chitosan's reaction with the salicyldehyde. The AuNPs was manufacture by extract of onion peels as a reducing agent. The Au NPs that have been prepared were characterized through the UV-vis spectroscopy, XRD analyses and SEM microscopy. The polymer blends of the chitosan Schiff base / PVP has been prepared through using the approach of solution casting. Chitosan Schiff base / PVP Au nano-composites was prepared. Nano composites and polymer blends have been characterized by FTIR which confirm the formation of Schiff base by revealing a new band of absorption at 1651cm^{-1} as a result of the (C=N) imine group. SEM, DSC and TGA confirms the thermal stability of the prepared polymer blends and nanocomposites, nano composites have shown good results in inhibiting esophageal cancer cell lines, IC_{50} of nanocomposite = 21.56 µg / mL.

Keywords: Anti-cancer cell line, Chitosan, Chitosan Schiff base, PVP, Nanocomposite.

Introduction:

Metal NPs/ polymer composite design had drawn a great deal of interest in the past few years, as a result of their broad variety of the applications, for instance, as catalysts and in bio-medical area[1]. Chitosan is a polysaccharide made from chitin that has been partially deacetylated [2]. Biocompatibility, safety, non-toxicity, moisture retention, antibacterial properties and ease of acquisition are only a few of the benefits of chitosan. In the realm of biomedicine, it is commonly employed. On the molecular chain of chitosan, there are several chemical groups that can participate in the reaction and can also be further changed as needed[3]. The primary amine's condensation with active carbonyl produces Schiff base complexes with an imine group (–RC=N). Its appeal as analytical reagents stems from the fact that they make determining various organic and inorganic compounds straightforward and affordable[4]. Functional groups inserted into the chitosan matrix could boost its ability to interact with the ions of the metal via complexation. In this regard, modifying the chitosan with the aldehydes in order to form Schiff bases might provide possibly complexing material for metallic species with environmental and analytical implications [5]. Polyvinylpyrrolidone (PVP) is a synthetic polymer with non-toxic, bioinert and hydrophilic qualities, making it a promising option for drug delivery applications in the pharmaceutical industry [6]. Polymer blends can be defined as the materials that are made through the blending of two polymer or copolymer types in order to produce a new material with complementary properties. Polymer mixing is an appealing strategy for developing new materials for particular uses since it is both cost-effective and simple [7].

Gold nanoparticles are one of the most widely utilized forms of metallic nanoparticles in different fields of research due to their biocompatibility, nontoxicity and ease of production. Recently, there has been a significant growth in the biological applications of gold nanoparticles, either alone or in combination with other types of nanoparticles in drug administration, photothermal therapy and as diagnostic instruments in a variety of medical applications [8]. Nanocomposites are

composites with nanoscale morphology such as nanoparticles, nanotubes, or lamellar nanostructures in one phase. They have multiphases and multiphasic materials should have at least one phase with diameters in the 10 –100 nm range. Nanocomposites have developed as useful alternatives to address the limitations of many engineering materials nowadays. Nanocomposites may be categorized based on their dispersed matrix and dispersed phase components [9]. Cancer can be described as malignant neoplasm disease which results due to the abnormal and uncontrolled cell growth that is the second main cause of death worldwide, after heart disease. Cancer represents the most dangerous type of cell proliferation, with heterogeneous abnormal cell growth that may localize within an affected organ (or invading to other body parts with process called metastasis) [10].

In the present study, onion peels extract were used to synthesize gold (AuNPs). Nanoparticles were characterized by UV-visible spectrophotometry, scanning electron microscopy (SEM), and XRD analysis. Polymer blends of the chitosan Schiff base / PVP have been prepared using solution casting method. Chitosan Schiff base / PVP Au nano-composites was prepared and characterized. The nanocomposite film showed significant anticancer activity against esophageal cancer cell line.

Materials and Methods:

Preparation of the Chitosan Schiff Base (Cs)

Chitosan 0.5 gm has been dissolved into 2% of acetic acid and heated for 1hour at 60 °C, while 2-hydroxybenzaldehyde 1 mL was dissolved in 10 mL ethanol. This mix has been magnetically stirred and heated at a temperature of 60 °C with reflex for 18 hours. The crude product was washed by the ethanol after cooling, and then dried in the oven at a temperature of 50°C for 24 hours [11]. Chitosan Schiff base's synthetic route is depicted in Fig.1.

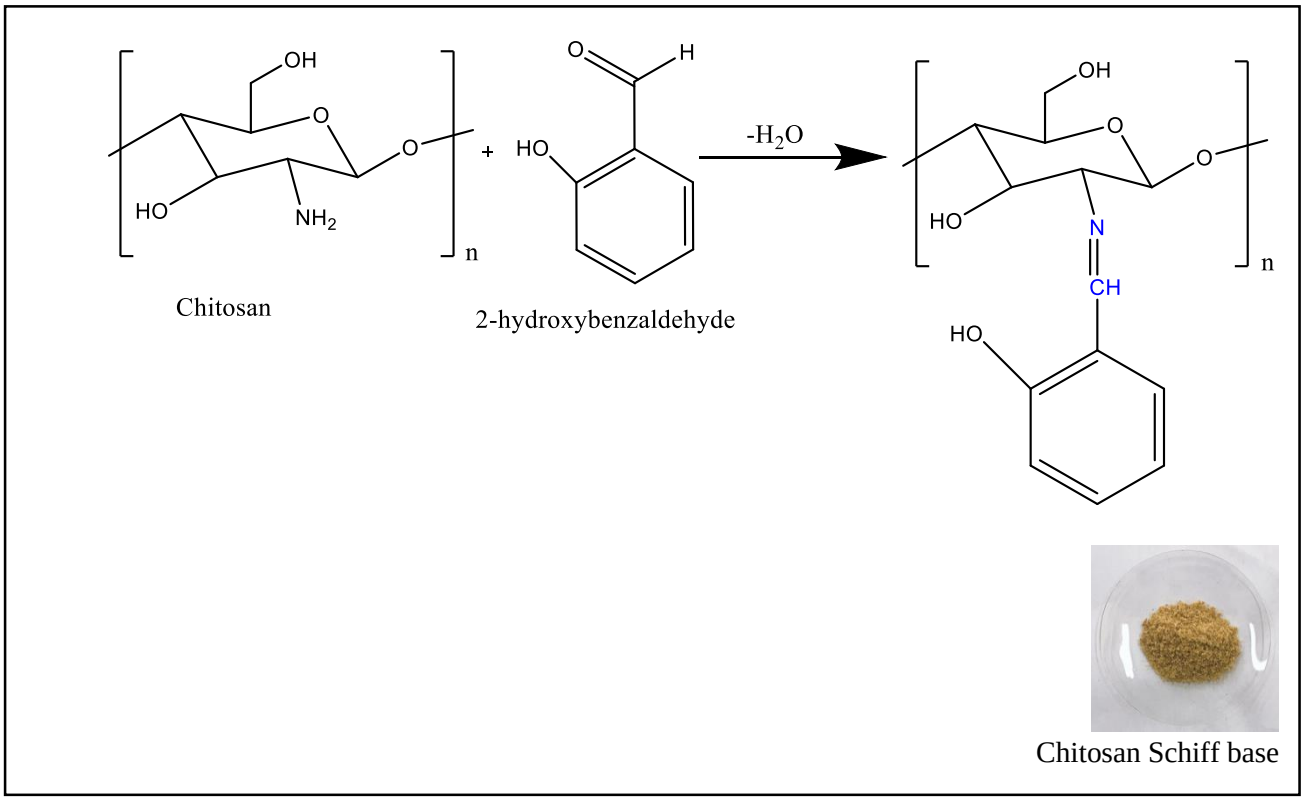


Figure 1. Synthesis of the Chitosan Schiff base

Polymer Blend Preparation

The chitosan Schiff base was prepared through dissolving 1gm of the chitosan Schiff base into 50 mL of 2% percent aqueous acetic acid solution and stirring for 1 hour at a temperature of 60°C, 5 gm PVP was dissolved into 50mL of the water in order to prepare 10% percent W / V polymer solutions, 10 mL Cs and 5 mL PVP polymer solutions were mixed in order to prepare homogenous solution by the use of hot-plate stirrer for 30min Cs / PVP mixes were made by combining different Cs: PVP ratios (10:5) [12].

Preparation of Crude Extract (Onion Peels)

Onion peels 10 gm was dissolved in 100 mL of deionized water to prepare onion peels extract. This mixture was stirred then heated at a temperature of 50°C for 2 hours and the resultant substances were filtered then dried in an oven at a temperature of 50°C, 0.02gm of the produced powder was dissolved in 100mL of the deionized water for the purpose of obtaining onion peels extract 200ppm, the fresh extract was obtained from the onion peels and utilized as a reducing and stabilizing agent [13].

Preparation Stock Solution of $HAuCl_4.3H_2O$

Gold chloride tri-hydrate ($HAuCl_4.3H_2O$) 1 gm has been dissolved ino 100mL of the deionized water for the purpose of preparing stock solution, then, 2mL has been obtained from the stock solution and filled to 50mL through applying the sequential operations of dilution to have 200ppm.

Green Synthesis of Gold NPs

The aqueous extract of the onion peels 3mL was added into 10mL of the aqueous solution of the gold chloride. The finished mixture was stirred for 10 minutes at a temperature of 25°C [14]. The gold colour altered from the yellow to purple, as shown in Fig. 2. indicating that AuNPs was formed. A centrifuge 10,000rpm was used for the purpose of separating nanoparticles from filtrate, after which the precipitate was taken, collected and diluted by the deionized water [15].

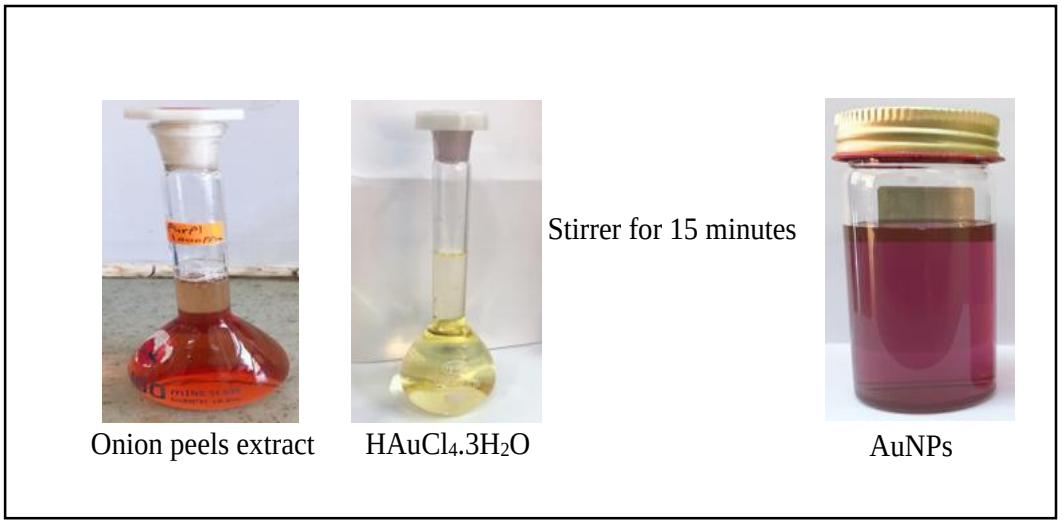


Figure 2.Synthesis of AuNPs

Preparing Chitosan Schiff Base (Cs) / PVP Au NPs

In order to make nanocomposite film, 10 mL of chitosan Schiff base, 5mL of the PVP, and 25mL of 2 different concentration values 75, 150 p.p.m. AuNPs were mixed and stirred for two hours. The mixed solution was then cast in Petri-dishes and stored in an oven of constant temperature at a temperature of 50°C for 24 hours [16].

Cell Cultures

SK-GT-4 cells have been cultured in RPMI1640 with 10% fetal bovine serum, 100g/mL streptomycin and 100units/mL penicillin. The cells have been passaged twice each week with the Trypsin-EDTA, then reseeded at a confluence of 80%, and incubated at temperature of 37°C [17, 18].

Cytotoxicity Assays

MTT test was utilized in 96-well plates in order to investigate cytotoxic impacts of nanocomposite [19, 20]. 1×10^4 cells per well were planted in each cell line. Cells were treated with nanocomposite after 24 hours or after confluent mono-layer has been established. After 48 hours of the treatment, cell viability has been determined through removing medium, adding 28L 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 the DMSO (Di-methyl Sulphoxide) and incubation at 37°C for 15 minutes with the shaking [21]. Absorbency was evaluated at 492nm with the use of micro-plate reader and the test was done in triplicate. The equation below was utilized in order to compute

cell growth inhibition rate (cytotoxicity percentage) [22, 23].

Rate of Inhibition = (A- B) / A×100

where A is the control optical density and B is the sample optical density [24].

Cells were seeded into 24-well micro-titration plates at a 1×10^5 cells 1mL density and cultured for 24 hours at 37ºC in order to examine their form under inverted microscope. After that, cells were exposed to the nano-composite for a period of 24 hours. Plates were stained by crystal violet dye then incubated at a temperature of 37ºC for 10–15 minutes after exposure duration [25]. The stain was carefully wiped away with the tap water to the point where all the color were gone. Cells were seen under a 100× magnification inverted microscope and photographs were taken using digital camera that has been mounted to microscope [26, 27].

Results:

UV– Vis Spectroscopy

The UV-Visible spectra of the AuNPs solution is illustreated in Fig.3. The unique surface plasmon resonance (SPR) absorption band of AuNPs emerges at 500 nm [28].

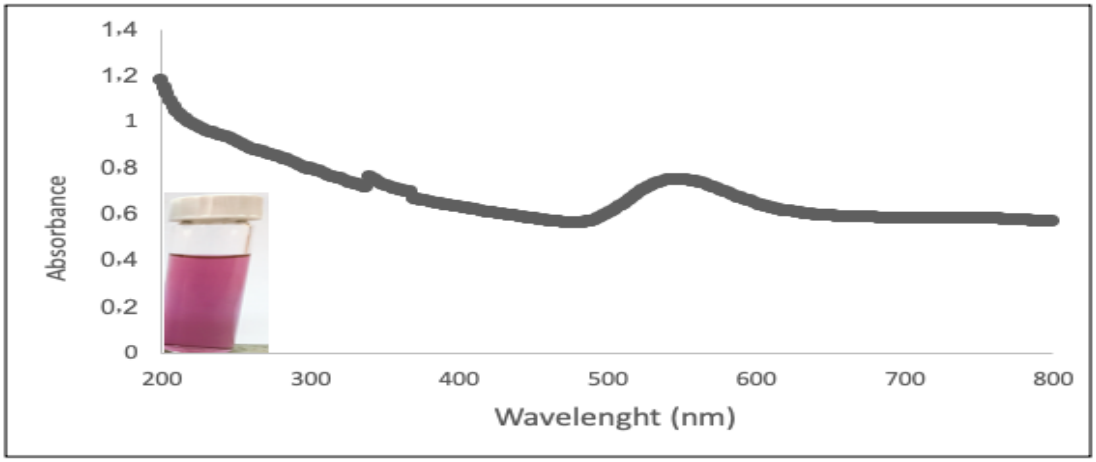


Figure 3. UV-vis spectrum of AuNPs

X-ray Diffraction Analysis (XRD)

The crystalline nature of produced AuNPs was determined with the XRD analysis. XRD data of AuNPs generated are shown in Fig.4 and Table. 1. At 10 and 80, AuNPs had a 2theta degree. According to AuNPs, sharp strong peaks were identified at 38º, 64º, 44º, and 77º, which matched to Braggs planes (111), (220), (200), and (311) planes were showed, confirming the AuNPs face-centered cubic (fcc) observed that peaks, respectively (JCPDS04-0784) [29].

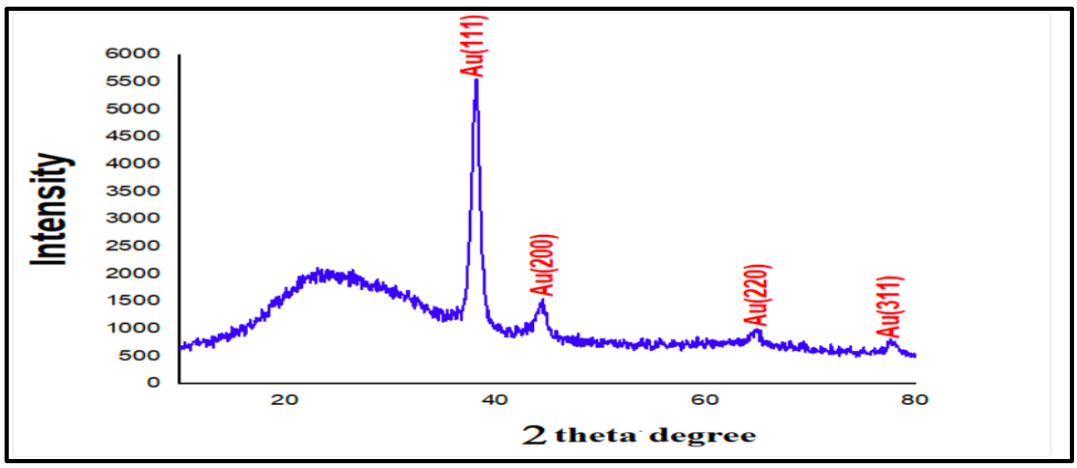


Figure 4. XRD Patterns of AuNPs

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

Element	2 theta	FWHM	Hkl	Average	D average
Au	38.2	0.59	111	3.11nm	
	44.4	0.49	200	4.12nm	
	64.9	0.78	220	4.27nm	3.96 nm
	77.7	0.78	311	4.36nm	

Chitosan Characterization, Chitosan Schiff Base, Blend and Nano-composites FT-IR Analyses:

Chitosan Schiff base Fig.5. A. revealed a new band of absorption at 1651cm^{-1} as a result of the (C=N) imine group, an absorption band at 1520cm^{-1} as a result of C=C of 2-hydroxy benzaldehyde, a peak at 759.96cm^{-1} attributed to aromatic range's C-H deformations due to (C–O–C) pairing stretching vibration in (1-4) glycosidic bonds, and additional peak values at 1060cm^{-1} Fig.5. B The existence of a hydroxyl (OH) group with the polymeric connection and (–NH) secondary amide is indicated by the band at 1014cm^{-1}. A band at 1423cm^{-1} is ascribed to pyridine ring (C=N) [30].

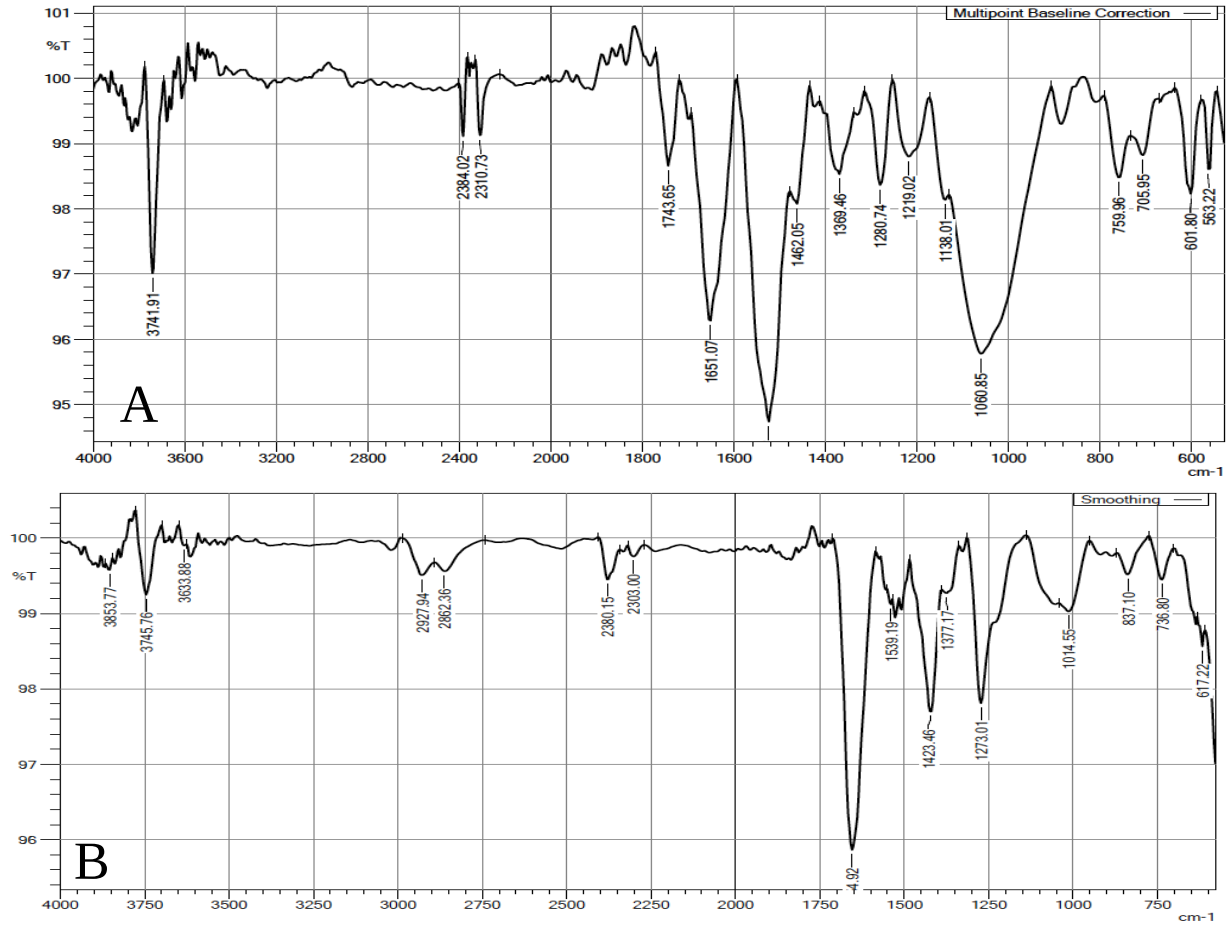


Figure 5. FTIR Spectrum A- chitosan Schiff base, B -Blend (Cs/PVP)

Scanning Electron Microscope (SEM)

Surface morphology, crystallinity, size, and phase locations of the produced material could all be researched by the examinations of SEM [31]. Surface morphology varies for Schiff base of the chitosan, polymer nano composite and polymer blends were investigated with the use of the SEM approach. The SEM images for pure gold nanoparticles are shown in Fig.6. D, the average nano size of the particles is ranged between 20 -70 nm for gold nanoparticles. The SEM 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 nanocomposite film were found with almost spherical morphology. However, some nanoparticles agglomerations were found on the rough surface of the nanocomposite. [32,33].

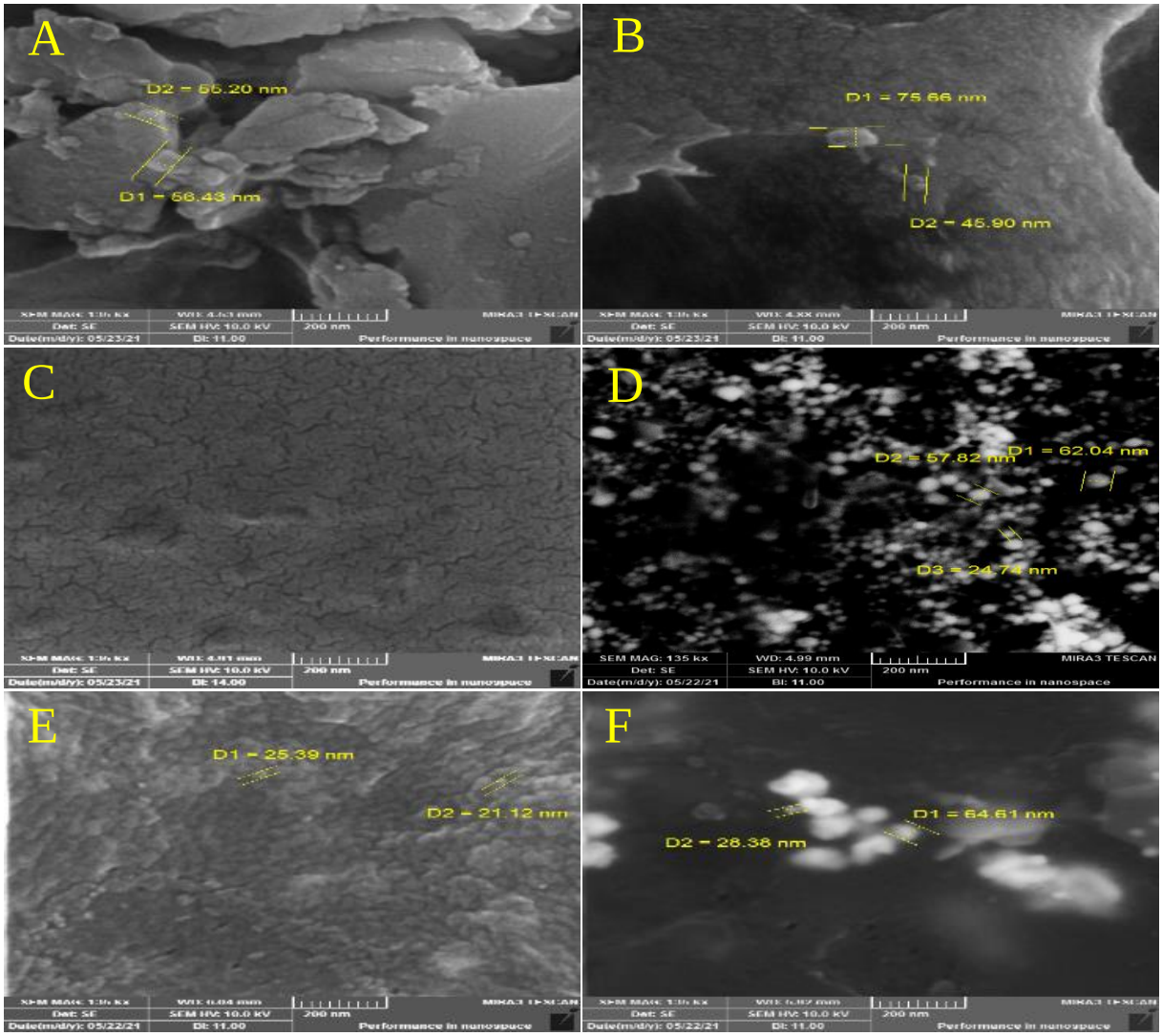
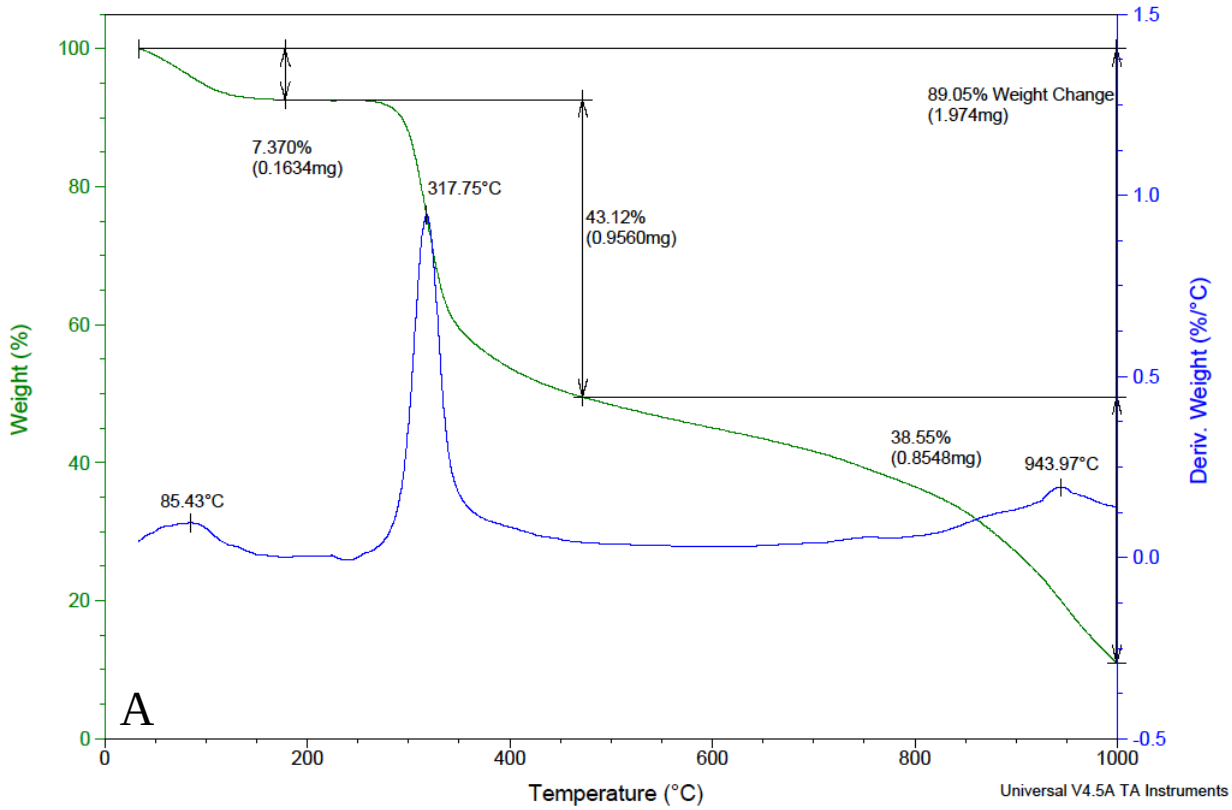


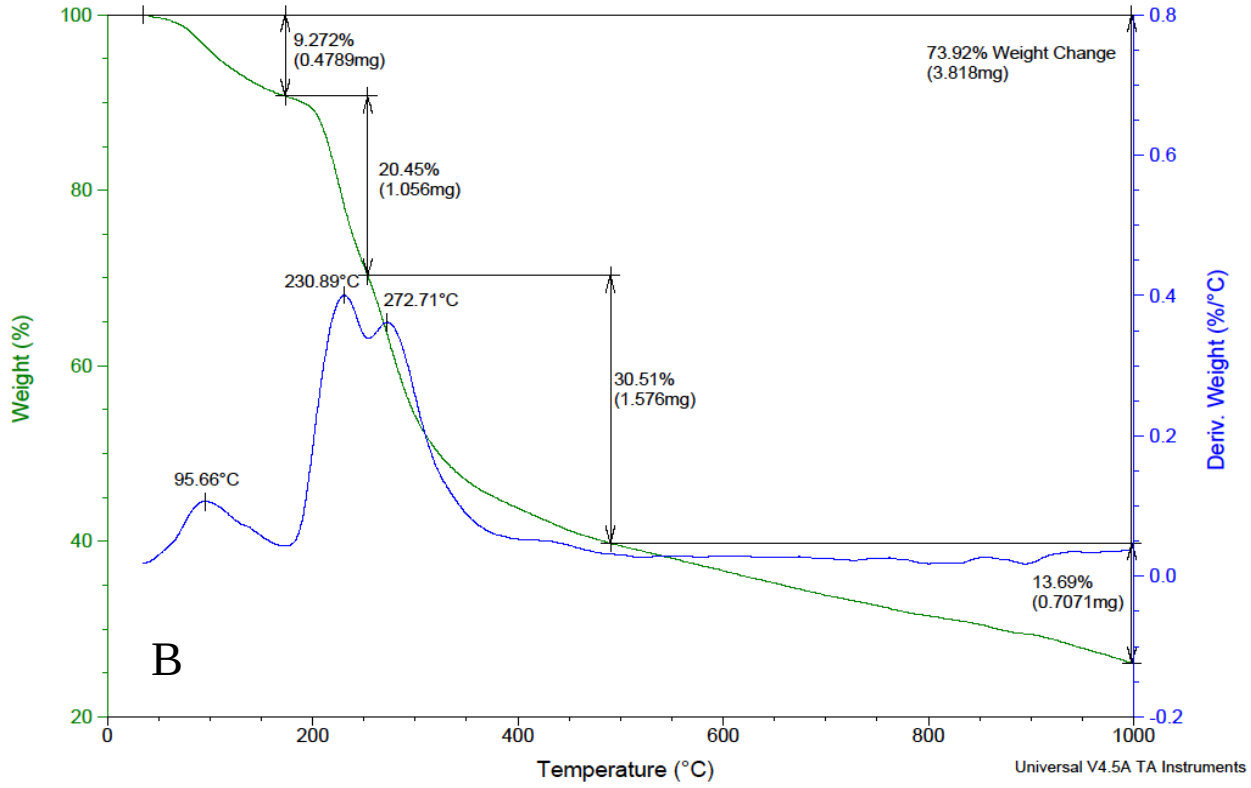
Figure 6. SEM image of A- chitosan, B- chitosan Schiff bases, C- Blend (chitosan/PVP), D- gold nanoparticles E- Nanocomposite (Cs/ PVP and AuNPs 75 PPM), F - Nano composite (Cs/ PVP and AuNPs 150 PPM).

Thermal Analysis

The thermal gravimetric (TGA, DSC) analysis of Cs, Cs Schiff base, Cs Schiff base / PVP polymer blend, and Cs Schiff base / PVP AuNP was carried out at temperatures ranging from 25°C to 1000°C at constant rate of 10°C minutes. The TGA curve of the Cs Fig.7. A illustrates 3 sequence mass loss stages. The curve of DSC in Fig.7. A for the Cs showed a Tg of (85.43°C). Peak at 317.75°C concerning polymer melting Tm . Cs Schiff base's TGA curve Fig.7. B illustrates 4 sequence mass lose stages. The curve of the DSC in Fig.7. B for the Cs Schiff base shows Tg of (95.66°C), peak concerning point of crystalline temperature Tc at 230.89°C. Peak at the temperature of 272.71°C concerning polymer melting Tm. The curve of the TGA of the Cs Schiff base / PVP polymer blend Fig.7 C, illustrates 4 sequence mass lose stages. The curve of the DSC in Fig.7 C for the Cs Schiff base / PVP polymer blend shows Tg of 85.06°C. The peak regarding crystalline temperature point Tc at (258.90°C). Peak value at a temperature of (445.02°C) concerning polymer melting Tm. TGA curve of Cs Schiff base / PVP-AuNP (NC-gold) Fig.7 D illustrates four sequence mass loss stages [34]. The curve of the DSC in Fig.7 D for the NC-Au shows Tg of the 101.26°C. Crystalline temperature point Tc at 266.96°C. Peak value at 448.25°C is concerning polymer melting Tm [35,36]. All temperatures were pushed a little higher. This higher thermal stability results from aldehyde derivative that was supported by NPs. This results in a reduced rate of thermal breakdown, demonstrating the effect of gold coordination bonding on thermal stability; also, the mix film only shows one Tg on its thermogram. This indicates that the blend has

hydrogen bonding connections between Cs and PVP and that the two polymers are effectively mixed. These results indicate that adding nano-Au to a Cs / PVP nanocomposite at such a low concentration can increase its thermal stability.





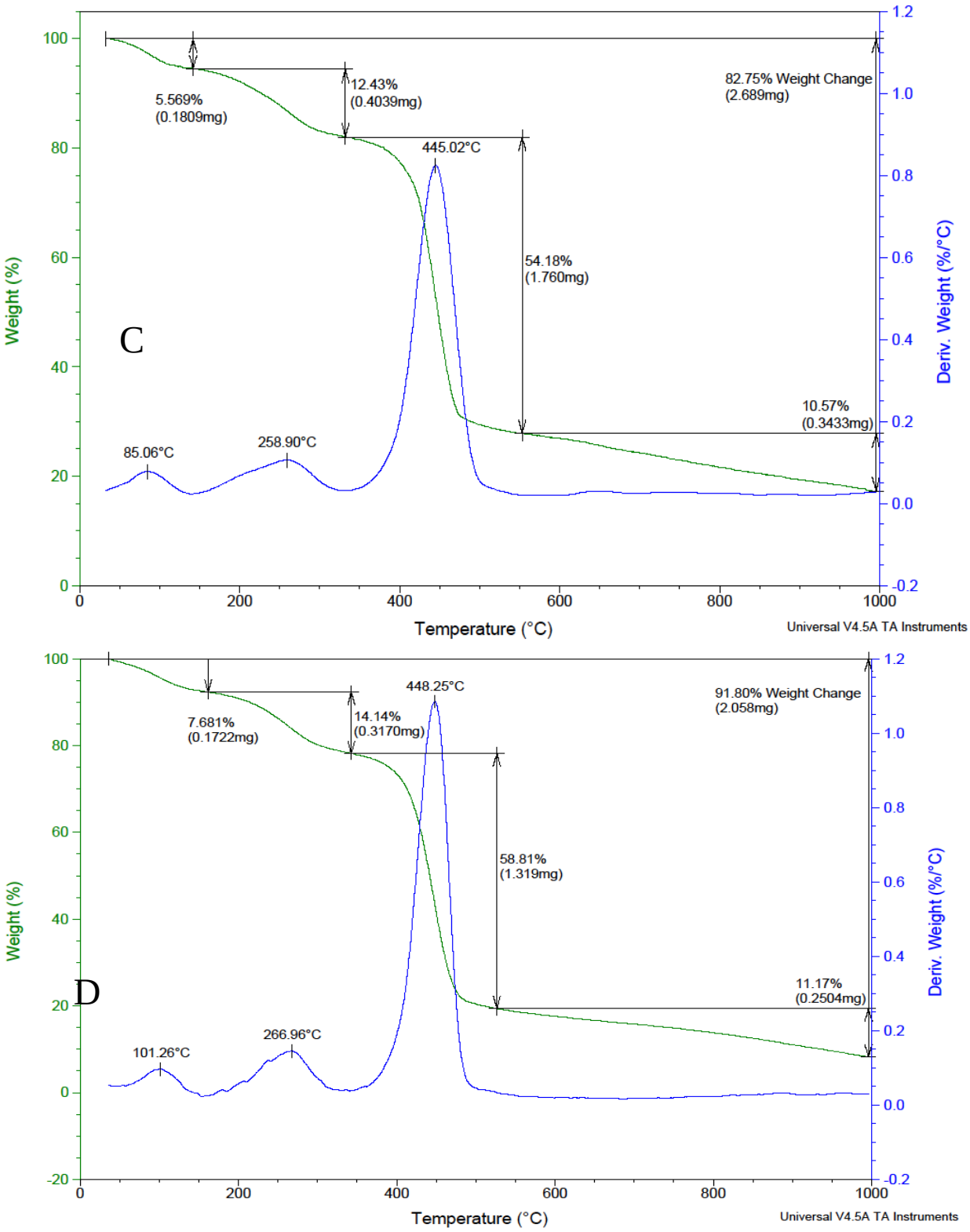


Figure 7. Thermal analyses (TGA and DSC) A- Cs, B- Cs Schiff base, C-Blend (chitosan/PVP), D-Nano-composite (Cs/ PVP and Au nanoparticles).

Disscusion:

Anti-cancer Cell Line

Cytotoxic effects of the nanocomposite against SK-GT-4 cells has been investigated. Anti-proliferative activity of nanocomposite have been tested through investigating their capability for inhibiting SK-GT-4 cell line proliferation. Results of this study are shown in Fig.8. The results demonstrated the ability of prepared nanocomposite to destroy and killed of cancer cells as shown in Fig.8. The activity of

nanocomposite against cancer cells is concentration dependent manner. Inhibition rate nanocomposite at concentration 3.1, 6.25, 12.5, 25, 50 µg/mL equal 7.33, 24.67, 37, 57, 80.67 respectively as shown in Fig.9. IC_{50} of nanocomposite=2I.56µg/mL[37].

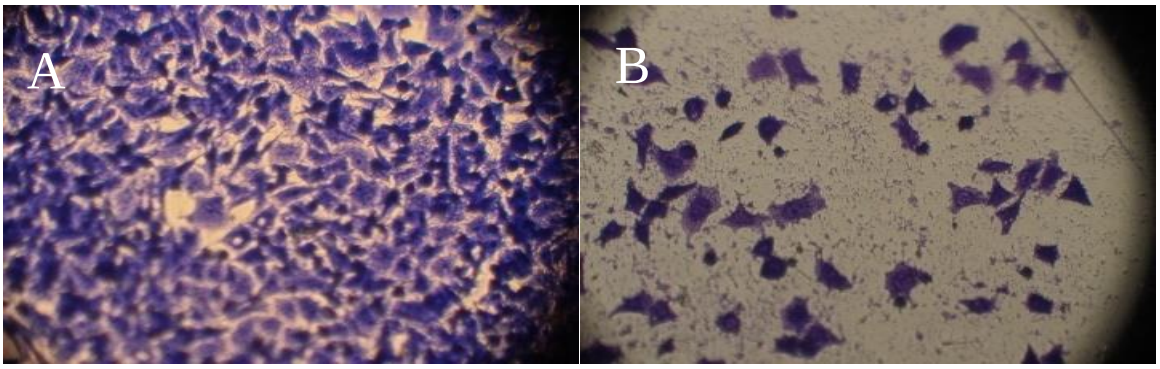


Figure 8. A- Control the untreated SK-GT4 cells, B- Morphological changes in the SK-GT4 cells after treated with nanocomposite

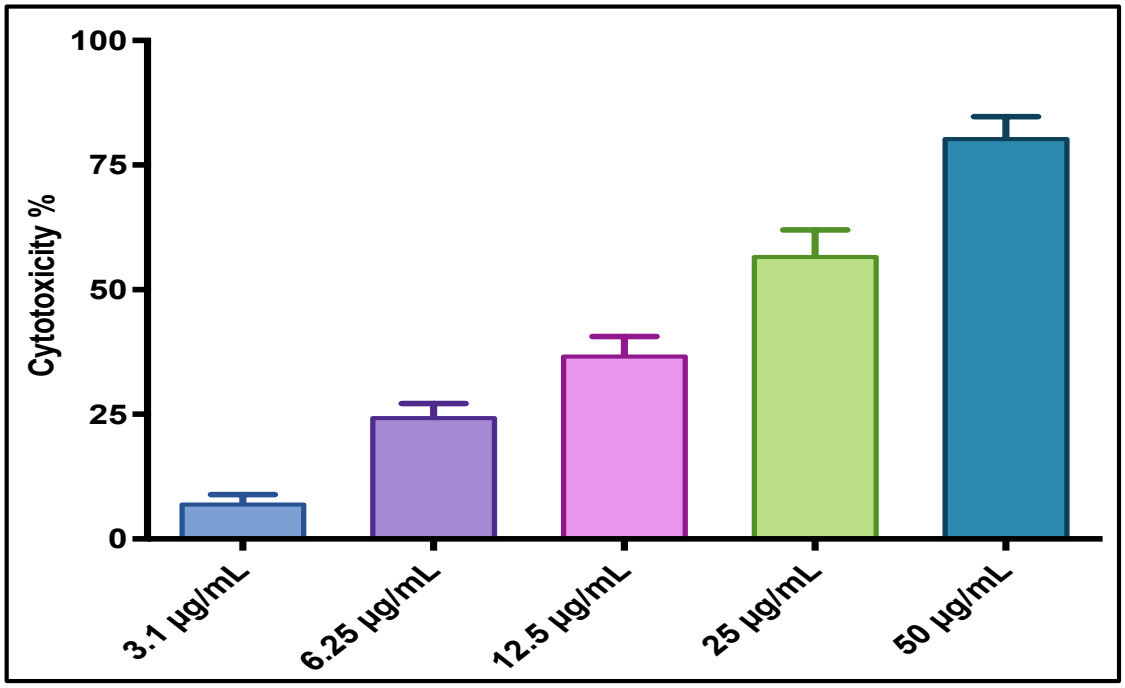


Figure 9. Cytotoxicity of nanocomposite in SK-GT-4 cells.

Conclusions:

In the current study, Cs/PVP blend was synthesized by solution casting method which could behave a nanoreactor for gold nanoparticle with promising anticancer applications. Cs/PVP/Au nanocomposite was confirmed by SEM, DSC and TGA analysis. The anti-cancer activity of the prepared nanocomposite was demonstrated in esophageal cancer cell line and it has a significant effect against esophageal cancer. SEM indicates that the nanoparticles of Au is noticed with the homogenous distribution on matrix surface. DSC analysis investigated the thermal stability of the polymer blend and nano composites illustrates sufficient thermal stability for all of the produced complexes and addition of nano gold improved thermal stability of chitosan Schiff base / PVP based composite. Adding AuNPs increases the biological activity. Due to the presence of Au nanoparticles, nanocomposites exhibited increased anticancer activity in esophageal cancer cell lines. SK-GT-4.

Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Besides, the Figures and images, which are not ours, have been given the permission for re-publication attached with the manuscript.
- The author has signed an animal welfare statement.

- Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad.

Authors' Contributions Statement:

M.A.Y. contributed to the design, the analysis of the results and to the writing of the manuscript. B.F. A. contributed to the implementation of the research, the analysis of the results and to the writing of the manuscript Both authors have read and agreed to final draft of the manuscript.

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تحضير,تشخيص والفعالية المضادة للسرطان لمتراكب الذهب النانوي لقاعدة شيف للكيتوسان/ بولي فاينيل بريليدون في معالجة الخط السرطاني للمريء.

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الخلاصة:
في الدراسة الحالية تم تحضير قاعدة شيف للكيتوسان من تفاعل الكيتوسان مع السلسلديهايد. تم تحضير دقائق الذهب النانوية بواسطة مستخلصات قشور البصل كعامل مختزل.شخصت دقائق الذهب النانوية المحضرة بواسطة مطيافية الاشعة فوق البنفسجية-المرئية ,مطيافية الامتصاص الذري وطيف حيود الاشعة السينية. تم تحضير خلائط قاعدة شيف للكيتوسان /بولي فاينيل بريليدون بطريقة خلط المحاليل. تم تحضير المتراكبات النانوية مع الذهب للخلائط المحضرة. شخصت الخلائط المحضرة والمتراكبات النانوية بواسطة مطيافية الاشعة تحت الحمراء والتي اكدت تكوين قاعدة شيف بظهور قمة مجموعة الإمين عند $1651cm^{-1}$ وبواسطة المجهر الالكتروني الماسح والتحاليل الحرارية التي اثبتت الاستقرارية الحرارية للمركبات المحضرة.اظهر المتراكب النانوي فعالية جيدة في تثبيط نمو خلايا المرئ السرطانية IC_{50} للمتراكب النانوي $= 21.56\ \mu g / mL$.

الكلمات المفتاحية: الخط المضاد للسرطان, الكيتوسان, قاعدة شيف للكيتوسان, بولي فاينيل بريليدون, المتراكب النانوي.