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Effect of the addition of silver nanoparticles on the biological activity of thiocarbohydrazide derivatives

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Abstract
Thiocarbohydrazide (TCH) derivatives [(II), (III) and (IV)] were prepared and characterized by X-RAY diffraction (XRD), infra-red spectrum (FTIR). Also silver nanoparticles was prepared by chemical reduction and characterized by X-RAY diffraction (XRD), infra-red spectrum (FTIR), atomic force microscope (AFM) and scanning electron microscope (SEM). Thiocarbohydrazide derivatives were functionalized with silver nanoparticles by sonication. The pure and functionalized TCH derivatives were used to study the biological activity against *Staphylococcus aureus* bacteria. The functionalized compounds with Ag nanoparticles have more Inhibition effect against *Staphylococcus aureus* than unfunctionalized compounds due to the small size and high surface area of Ag nanoparticles.

Key words: Thiocarbohydrazide derivatives, Silver nanoparticles, SEM, XRD, Biological activity.

1. Introduction
Nanotechnology is a new type of science that refers to understand and control the properties of materials at the nano-scale, one nano-meter (one billionth of meter) is the dimension of a small molecule. At this level, matter exhibits diverse and often amazing properties and the borders between established scientific and technical disciplines fade [(1)]. Nanoparticles are a nano structures have a wide class of substances (metals, semiconductors, superconductors, magnetic materials, biomaterials, polymers, water soluble inorganic and organic compounds [(2)]. All these types of nanoparticles (NPs) are a materials with sizes vary between the order of one nanometer to several tens or hundreds of nanometers. Some times more than 100 nano as found in nano cluster[(3)] with many variable features as size, shape, surface charge and narrow size distribution[(4)] .

One of the important nanoparticle is silver nanoparticles which have widely and significance antibacterial effect. The amazing features of these articles require a large number of pathogenic bacteria, including antibiotic-resistant strains, as well as many viruses and parasites. The nano silver has an antibacterial pesticide longer the privilege of a deadly virus, terrible natural antibiotic and several miracle cures source [(5-10)].

The medical properties of silver have been known for many years. Silver-based compounds are used in many antimicrobial applications. Nanoparticles have been known to be used for many physical, biological, and pharmaceutical applications therefore, many researches have found that using silver nanoparticles as pharmaceutical or using it as base material to prepare many drugs [(11)].

Nanoparticles-based compounds usually have better or different qualities as antibacterial than the bulk material of the same element. This could be attributed to high size to volume ratio which found in nanomaterials. In the case of silver nanoparticles-based compounds the antibacterial effect is greatly enhanced due to tiny size that causes immense surface area relative to volume. Therefore very small amounts of silver Nanoparticles-based compounds can lend antimicrobial effects to hundreds of square meters of its host material [(7)].

2- Experimental part
2.1. Materials:
All chemicals including hydrochloric acid (HCl), silver nitrate ($AgNO_3$), hydrazine monohydrate ($N_2H_4.H_2O$), carbon disulphide (CS_2), pyridine (C_4H_4N), sodium triacetate, trephthaldehyde, phthalic anhydride,were of analytical grade are used as received without further purification except aniline.

2.2 .Instrumentation:
All infra-red spectra of nanomaterials were recorded by (65 FT-IR Perkin Elmer Spectrophotometer). The prepared materials were characterized by x-ray diffraction using (Shemadzu – XR – 6000) device with Nickel - Copper filter for the x-ray radiation (Cu Kα, λ = 1.5406 Å). Atomic Force Microscope (AFM) type PHYWE was used to study the surface of the nanoparticles.

2.3 .1 Preparation of Materials:
2.3 .1. Preparation of Silver Nanoparticle:
Silver nitrate solution of 0.17 g of $AgNO_3$ in 100 ml of distilled water was boiled and 0.103 g of sodium acetate in 10 ml of distilled water was added to it [(12-14)].

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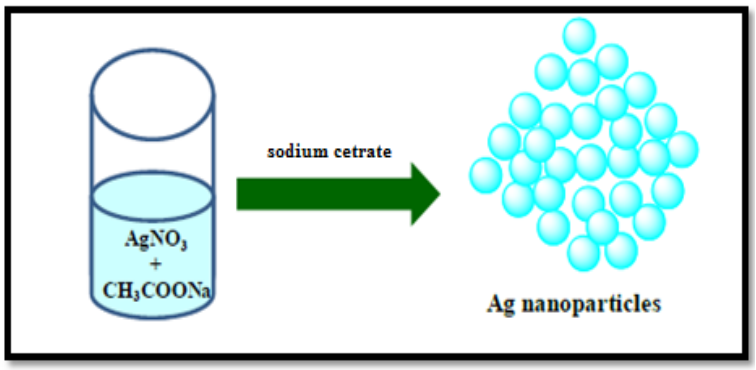

Fig (1): Preparation of Silver Nanoparticle

The equation of reaction could be expressed as follows:

$$4\,Ag^{+} + C_6H_5O_7Na_3 + 2\,H_2O \rightarrow 4\,Ag^{0} + C_6H_5O_7H_3 + 3\,Na^{+} + H^{+} + O_2\uparrow$$

Equation (1): Preparation of Silver Nanoparticle

2.3.2 Preparation of compound (I):
20 ml of hydrazine hydrate was added drop wise to 5 ml of carbon disulphide (CS_2). This mixture was refluxed for 30 minutes, until yellow-white precipitate was formed. The yellow-white precipitate was washed in ethanol, recrystallized in distilled water until white crystals were formed, dried it in 70 ^{O}C for 4 hours [15, 16].

2.3.3. Preparation of compound (II):
A mixture of 1.06 g of TCH and 1.44 g of CS_2 were refluxed with 10 ml of pyridine for 2 hours. Then the solution was kept in the room temperature for 1 hour. The precipitate was pale yellow prisms recrystallized from warm water. The products was pyridinium salt which crystal.

1 g of pyridinium salt was dissolved in 10 ml in hot distill water and 1.5 ml of concentration. HCl was added drop wise and the mixture was stirred 30 minutes and filtered, recrystallized in 1N HCl until yellow crystals were formed, dried at 70^{O} C for 4 hours.

2.3.4. Preparation of compound (III):
The ethanolic solution of compound (II) (0.58 g in 15 ml), was mixed with the ethanolic solution of trephthaldehyde (0.67 g in 15 ml) acidified with three drops of glacial acetic acid and refluxed for 5 hours at 75˚c and left overnight. The product was filtered and washed by water, dried at 70˚c for 4 hours**.**

2.3.5. Preparation of compound (IV):
0.348 g of compound (II) and 0.444 g of phthalic anhydride were refluxed in (25 ml) of glacial acetic acid for 5 hours, then the solution was kept at room temperature overnight. The product was filtered, washed by water and dried at 70˚C for 4 hours**.**

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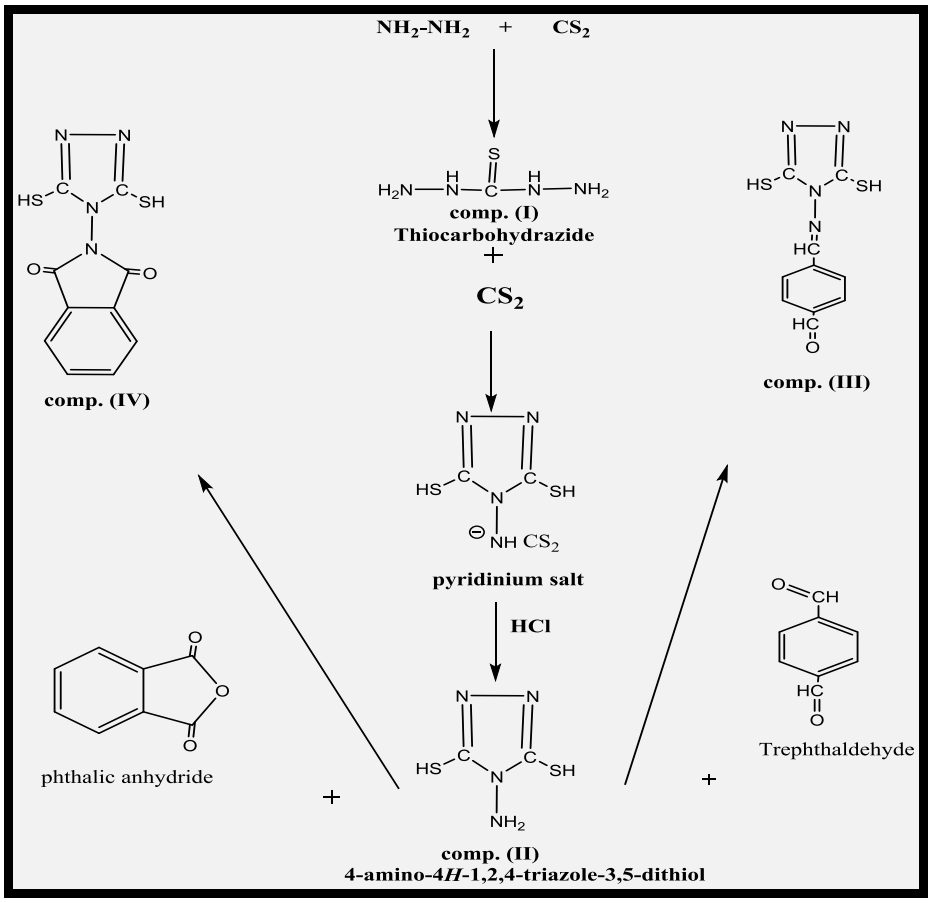

Scheme (1): preparation of comp. [(I) ,(II), (III) and (IV)]

2.3.6. Preparation of [compound (II), compound (III) and compound (IV)] functionalized with Silver nanoparticles:

0.5 g of compound (II) dissolved in 25 ml of ethanol with stirring; 0.05 g of silver nanoparticles was added to the above solution. The mixture was sonicated for 10 hours; the product was filtered directly and left the solute 3 days to remove the solvent. The yield is compound (II) functionalized with Silver nanoparticles.

Compound (III) and compound (IV) functionalized with Silver nanoparticles were prepared according to the method in (2.3.6).

3. Results and discussion

3.1. FTIR characterization:

3.1.1. FTIR of Thiocarbohydrazide (compound I):

Thiocarbohydrazide spectrum Figure 2 showed peaks at 1531,755 and 1490cm^{-1} which are associated for N-H wagging, bending and C-N stretching vibration, respectively. The bands of the characteristic (C=S) stretching were observed at 1286 and 933 cm^{-1}. The band in 3305 cm^{-1} is due to N-H stretching vibration, and the bands at 3274 and 3204 cm^{-1} are due to NH_2 stretching vibrations. 1639 and 1142 cm^{-1} bands are assigned to the NH_2 bending and wagging vibrations [17, 18].

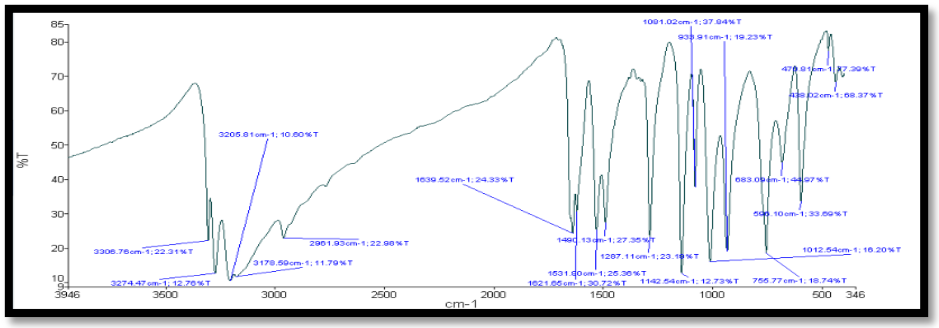

Fig (2): FTIR of comp. (I).

3.1.2. FTIR of compound (II) and compound (II) functionalized with Silver nanoparticles:

The infrared spectra of these compounds show in figure 3. The double band at 3278, 3146 cm^{-1} assigned toNH_2 stretching vibration. These diagnostic bands are:

1608 cm^{-1} and 1259 cm^{-1} assigned to NH_2 bend and C-N stretching vibration respectively. Weak band

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at1458 cm^{-1} assigned to N-N cm^{-1} stretching vibration, while the band at 2791 cm^{-1} is attributed to the mercaptan group (S-H).

On the other hand, the change in NH_2 beaks sites and decrease the intensity of all beaks shows that the silver nanoparticles was coordinate with the organic compound.

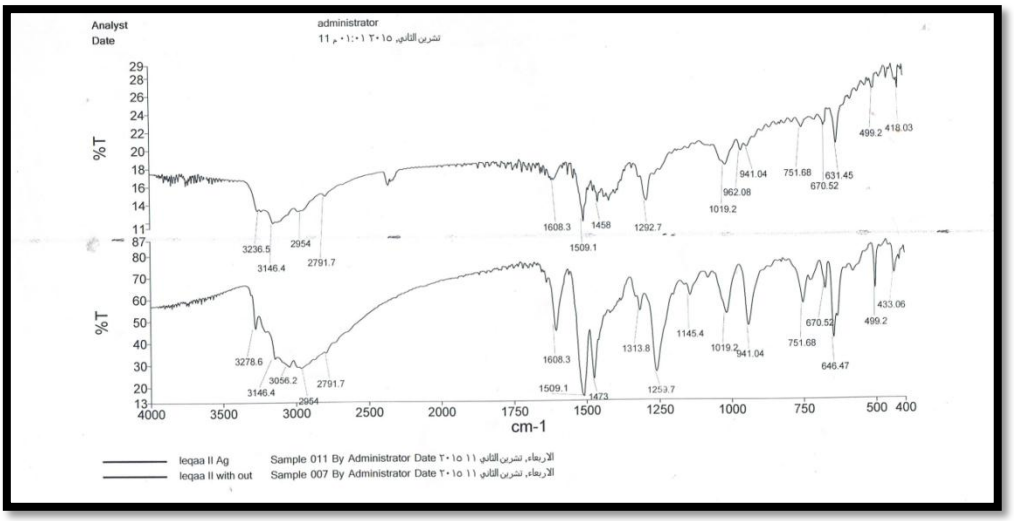

Fig (3): FTIR of compound (II) and compound (II) with Ag NPs.

3.1.2. FTIR of compound (III) and compound (III) functionalized with Silver nanoparticles:

Figure 4 shows the peak at 2752 cm^{-1} corresponding to the vibrations of S-H and 1602 cm^{-1} is assigned to the stretching of C=N. While the band at 835 cm^{-1} is attributed to the bending vibration of C-H aromatic ring. The bands at 3278 and 3146 cm^{-1} of NH_2 disappear, moreover, the new bands at 3152 and 1671 cm^{-1} are attributed to the N-H and C=O respectively .

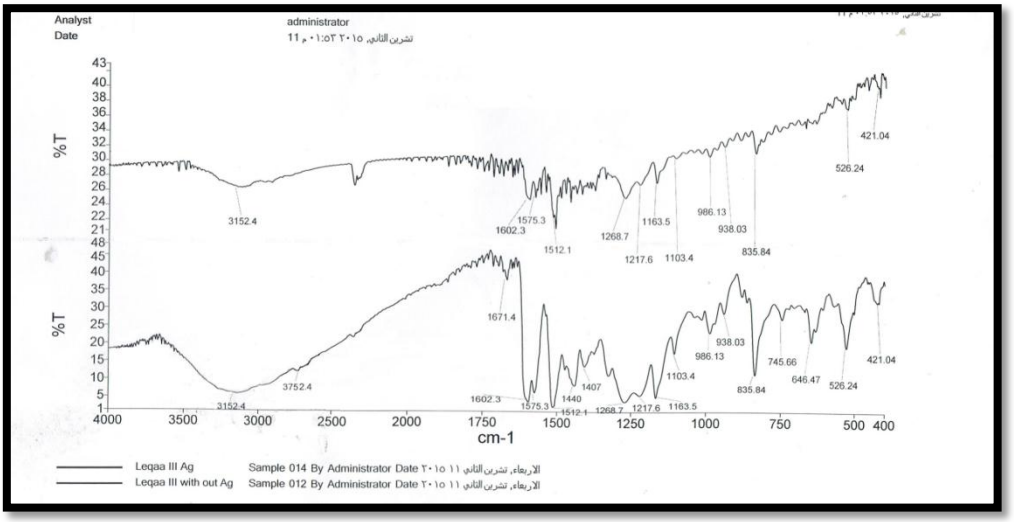

Fig (4): FTIR of comp. (III) and comp. (III) with Ag NPs.

3.1.2. FTIR of comp. (IV) and comp. (IV) Functionalized with Silver nanoparticles:

Figure 5 show bands at 3278 and 3146 cm^{-1} of NH_2 disappear, moreover, the new bands at 1605 and 1701 cm^{-1} are attributed to the N-N and C=O, respectively.

The band at 2791 cm^{-1} is attributed to the mercaptan group (S-H), the absorption peak at 1262 cm^{-1} are correspond to the stretching vibrations of C-O of group.

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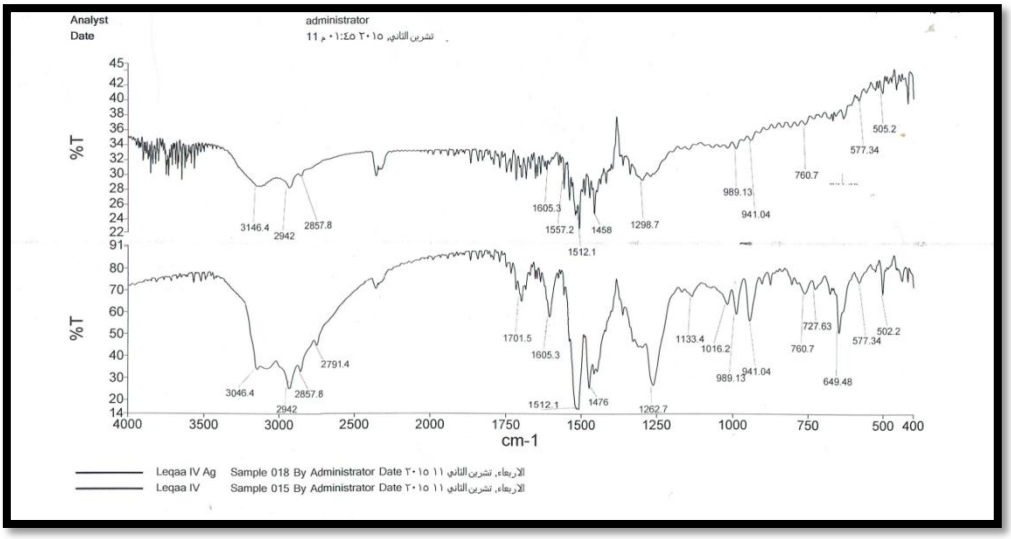

Fig (5): FTIR of comp. (IV) and comp. (IV) with Ag NPs.

3.2. X-Ray Diffraction Characterization (XRD):

3.2.1. XRD of Silver nanoparticles:

Figure 6 shows the X-ray diffraction pattern of silver nanoparticles. The diffraction pattern shows four sharp and well defined diffraction lines at 2θ = 37.6°, 43.34°, 65.5° and 76.3.From the peaks at 2θ = 37.6°, by using Deby- Scherrer equation, the average size was found to be 46.3 nm[19,20] .

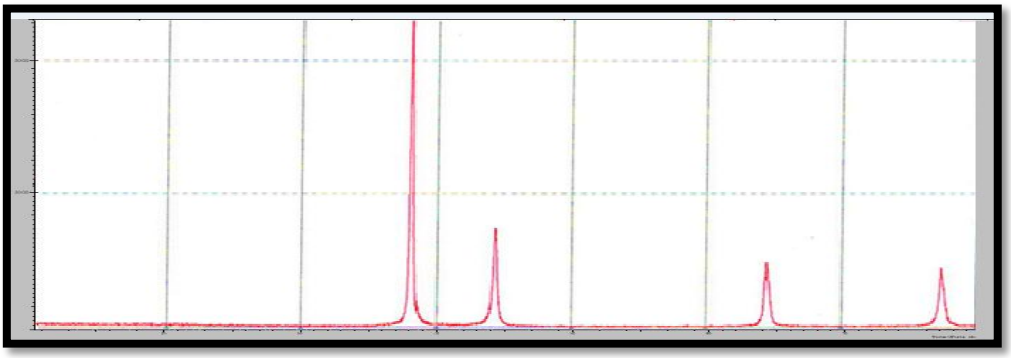

Fig (6): XRD of Silver nanoparticles

3.2.2. XRD of [compound (II), compound (III) and compound (IV)] functionalized with Silver nanoparticles:

Throughout XRD characterization in figures 7, 8 and 9, silver nanoparticles peaks were in the same region but with different intensities. In addition, new peaks were noticed. This could explain the formation of Ag-thiocarbohydrazide derivatives and indicate the formation of new-Thiocarbohydrazide derivatives with silver nanoparticles.

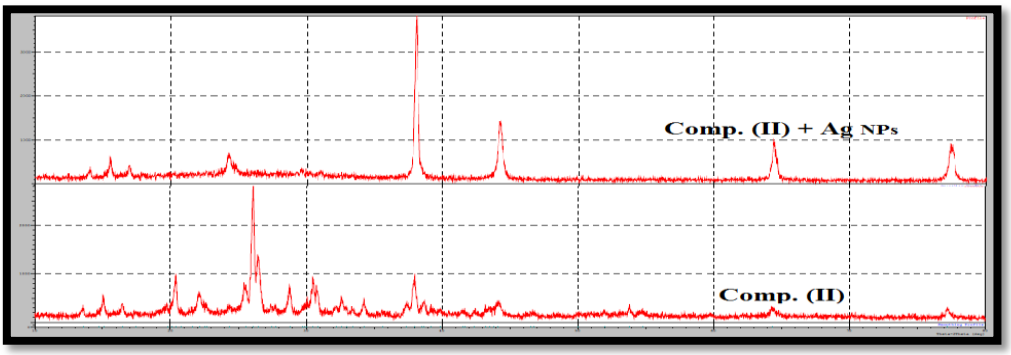

Fig (7): X-RD of comp. (II) Functionalized with Silver nanoparticles

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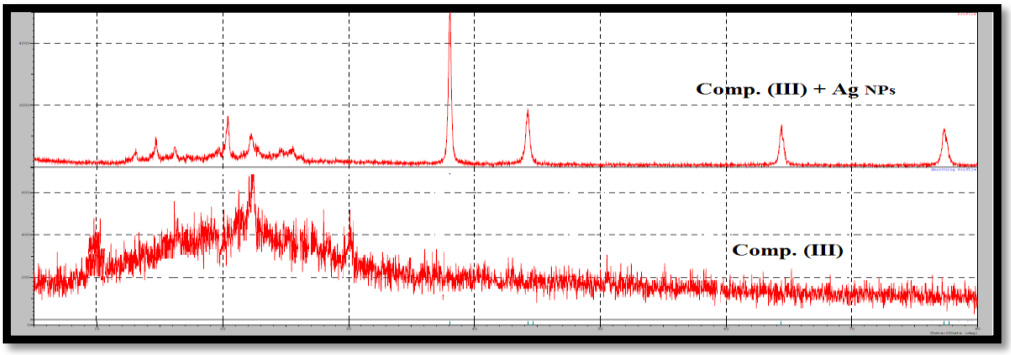

Fig (8): X-RD of comp. (III) Functionalized with Silver nanoparticles

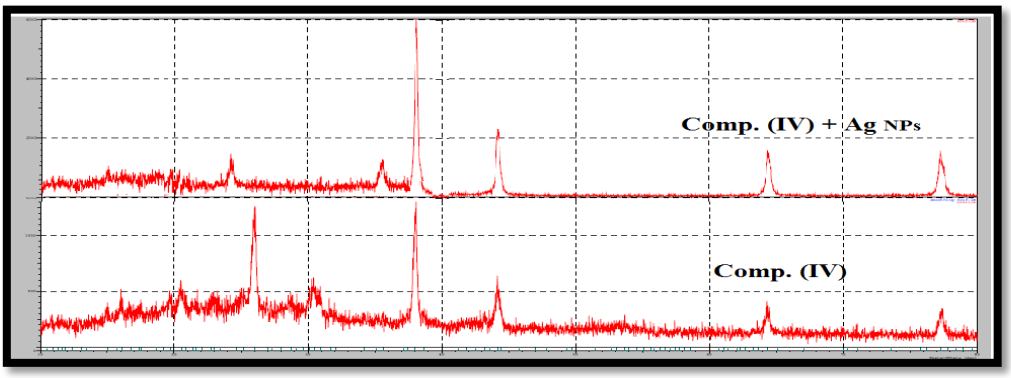

Fig (9): X-RD of comp. (IV) Functionalized with Silver nanoparticles

3.3 Atomic Force Microscope (AFM):-

It is important to study the topography of the materials in nanoscale and to know the morphology and surfaces for the nanoparticles. AFM investigations of pure silver nanoparticles in figure [10]. The AFM measurements show the size equal to 43.1 nm of the region.

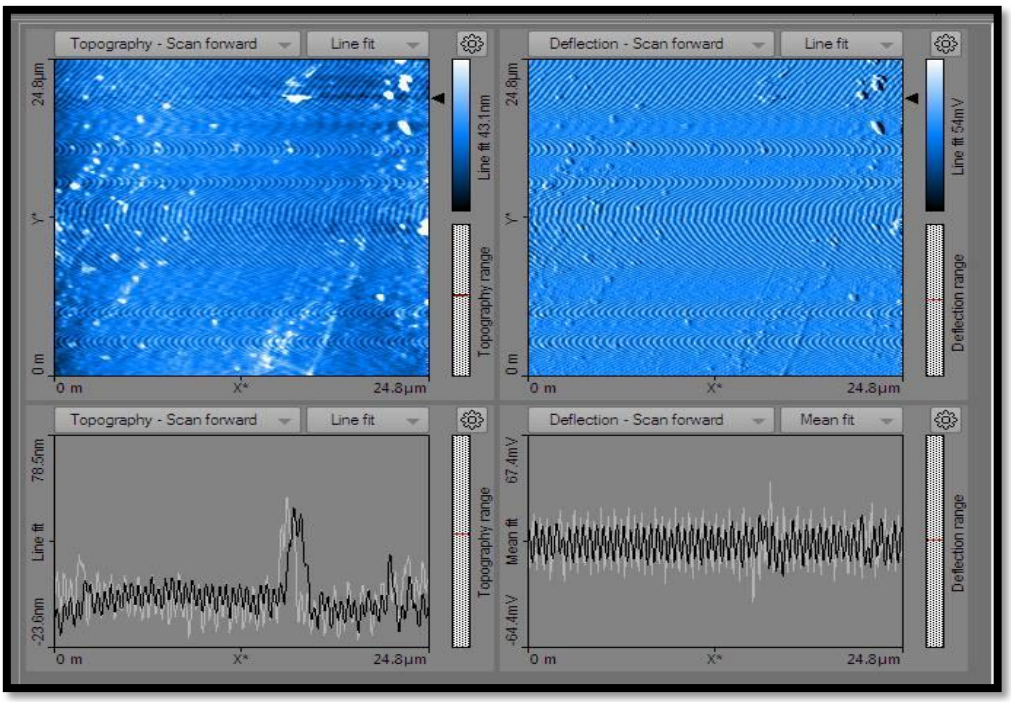

Fig (10): AFM image of silver nanoparticles.

3.4- Scanning electron Microscope (SEM):

The scanning electron microscopy shows the following images in micro scale for silver nanoparticles.

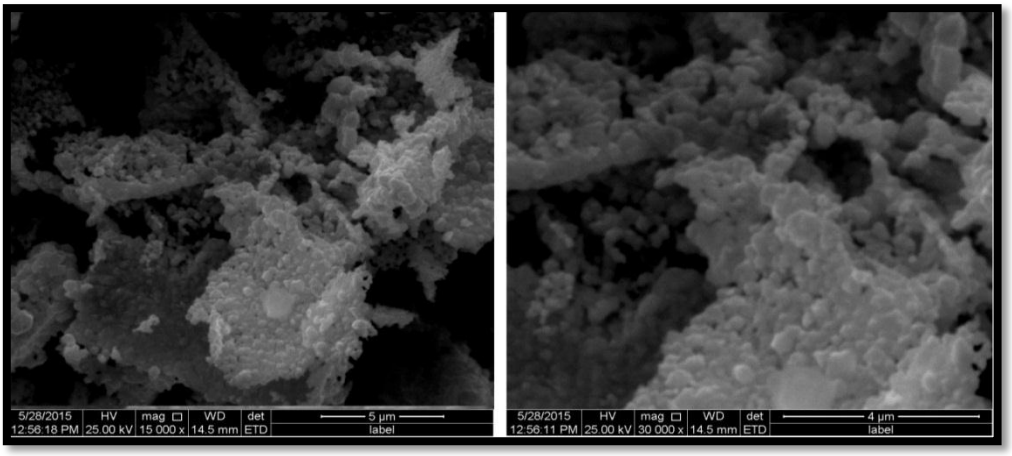

Fig (11): SEM images of silver nanoparticles in microscale

4. The Biological Activity

These compounds were dissolved in 5 ml DMSO in order to prepare 0.2 M to study the inhibition biological activity against *Staphylococcus aureus*. The biological activities have been studied using [compound (II), compound (III) and compound (IV) dissolved in DMSO] and its composites with Ag nanoparticles after dissolved in DMSO. The functionalized compounds with Ag nanoparticles gave good positive result against *Staphylococcus aureus* than unfunctionalized compounds as shown in table 1. These results indicate that the Ag nanoparticles promote the biological activity of organic compounds and this could be attributed to the small size and high surface area [21-25].

Table (1): The results of the biological activity of organic compound and their derivatives with Ag nanoparticles.

No.	Compounds	Distance (mm)
1	DMSO	----
2	compound (II)	9.5
3	compound (III)	7
4	compound (IV)	10
5	Compound (II) with Ag NPs	18
6	Compound (III) with Ag NPs	23
7	Compound (IV) with Ag NPs	27.4

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تأثير إضافة دقائق الفضة النانوية على الفعالية البايلوجية لمشتقات الثايوكاربوهيدرازايد

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قسم الكيمياء ، كلية العلوم ، جامعة ديالى ، ديالى ، العراق

الملخص

حُضرت مشتقات الثايوكاربوهيدرازايد [(II), (III) and (IV)] وتم تشخيص هذه المركبات بتقنية الاشعة تحت الحمراء وبتقنية حيود الأشعة السينية. كما تم تحضير دقائق الفضة النانوية بالاختزال الكيميائي وقد شخصت هذه الدقائق بتقنيات حيود الاشعة السينية ومجهر القوة الذرية والمجهر الالكتروني الماسح. وقد تم مفاعلة المركبات الثلاثة على دقائق الفضة النانوية عن طريق الموجات فوق الصوتية ثم استخدمت هذه المركبات النقية و المحتوية على الفضة النانوية في دراسة الفعالية البايلوجية ضد بكتريا المكورات العنقودية (*Staphylococcus aureus*) وقد وجد بأن المركبات المحتوية على الفضة النانوية هي الاكثر تثبيط لهذه البكتريا وذلك كونها حاوية على دقائق الفضة النانوية التي تمتاز بصغر حجم هذه الدقائق ومساحتها السطحية العالية.

الكلمات الدلالية: مشتقات الثايوكاربوهيدرازايد, دقائق الفضة النانوية, حيود الاشعة السينية, الفعالية البايلوجية.