

PLASMONIC ENHANCEMENT IN MOLECULAR FLUORESCENCE OF NATURAL CURCUMIN DYE WITH SILVER NANOPARTICLES SYNTHESIS & BIOLOGICAL APPLICATION

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Abstract

In this study, silver nanoparticles were prepared using the liquid pulse laser ablation technique. To target pure silver immersed in distilled water, a pulsed laser (Nd: YAG) with a wavelength of 1064 nm, number of pulses 600, constant ablation energy 530 mJ and frequency 1Hz was used. The effect of adding curcumin at certain concentrations to silver nanoparticles on the optical and structural properties was studied, which included UV spectroscopy, fluorescence spectroscopy, X-ray diffraction (XRD), and electron field emission (FESEM) microscope and Fourier transform infrared spectrometry. The (FTIR) and biological activity of silver nanoparticles and curcumin were tested on two types of bacteria. The results indicated that the increase in curcumin concentration was the reason for the improvement of visual properties. For silver nanoparticles and increase the surface plasmon resonance intensity, thus increasing the formation of nanoparticles, as well as improving the fluorescence. The infrared Fourier test of silver nanoparticles has been studied, as the absorption that appears near wave number (3451) cm^{-1} is caused by the vibration of the (OH) bond of the alcohol group. The absorption band 2927 cm^{-1} is the aromatic (C-H) bond, and this confirms that curcumin acts as a cover agent. An X-ray diffraction study of silver nanoparticles and a hybrid of silver nanoparticles and curcumin was performed. The X-ray diffraction results of silver nanoparticles showed that the crystal system is cubic and polycrystalline, and several angles that are completely identical to the international standard cards for silver were recorded. In this study we studied the biological application on two types of bacteria (*E. Coli*, *Staphylococcus aureus*) and the results showed that the highest bacterial inhibition was in the hybrid sample.

Keywords: Plasmonic, Fluorescence, Silver nanoparticles, Curcumin, Bacteria, X-Ray diffraction.

1. INTRODUCTION

PLAL is a promising technology because it can manufacture a wide range of nanoparticles, including metals, noble metals, semiconductor insulators, nano alloys, and oxides. It also has a number of advantages, including a low-cost method and one-step, high-purity highly crystallized nanomaterials can simply be obtained due to the use of only water and the target (Ali & Raouf 2011) and it is Clean and simple preparation compared to the chemical process that pollutes the final product and the environment (Huang & El-Sayed 2010).

The nanoparticles can easily be obtained well in one step without subsequent heat treatment due to the high active state of the exotic species (Xiao *et al.* 2017). It is possible to manufacture two or more materials at the same time (Haider & Mehdi 2014).

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2. ADVANTAGES OF THE PLAL METHOD

PLAL is a promising technology because it can manufacture a wide range of nanoparticles, including metals, noble metals, semiconductor insulators, nano alloys, and oxides. It also has a number of advantages, including:

1. A low-cost method and one-step, high-purity highly crystallized nanomaterials can simply be obtained due to the use of only water and the target (Ali & Raouf 2011).
2. Clean and simple preparation compared to the chemical process that pollutes the final product and the environment (Huang & El-Sayed 2010).
3. Due to the high active state it is easy to obtain nanoparticles well in one step without subsequent heat treatment (Xiao *et al.* 2017).
4. It is possible to manufacture two or more materials at the same time (Haider & Mehdi 2014).

3. SILVER NANO PARTICAL:

Metallic nanoparticles have been used in wide applications in different fields. Since the shapes, sizes and compositions of metallic nanomaterials are closely related to their physical, chemical and optical properties, nanomaterials-based technologies have been exploited in a variety of fields from chemistry to medicine (Millstone *et al.* 2009), (Lee *et al.* 2018), (Lee *et al.* 2012). Silver nanoparticles (AgNPs) have recently been extensively investigated due to their superior physical, chemical and biological properties, and their superiority mainly stems from the size, shape, composition, crystallinity and structure of AgNPs compared to their bulk forms (Syafiuddin *et al.* 2017), (Kumar *et al.* 2008), (Desireddy *et al.* 2013), (Sun & Xia 2002), (Atwater & Polman 2011). Efforts have been made to explore their attractive properties and use them in practical applications, such as antibacterial and anticancer therapies (Le Ouay & Stellacci 2015), diagnostics and optoelectronics (Chen *et al.* 2009), (Sun *et al.* 2003), (Haes & Van Duyne 2002) water disinfection (Dankovich & Gray 2011) and other clinical therapies/pharmaceutical applications (Zhang *et al.* 2016). Silver has great physical properties and is a low cost and abundant natural resource. Previous discoveries showed that the physical, optical and catalytic properties of AgNPs are strongly influenced by their size, distribution, morphology and surface properties that can be modified by different synthetic methods, reducing agents and stabilizers (Sun & Xia 2002).

4. CURCUMIN:

The main component derived from dried turmeric roots is curcumin, a hydrophobic polyphenol. It is a powerful antioxidant and anti-inflammatory and has a wide range of

pharmacological properties, the formula ($C_{21}H_{20}O_6$). It is also safe at doses of up to 8 grams per day, according to various studies; However, the oral bioavailability of curcumin is poor due to physiological pH instability, low water solubility, and rapid metabolism. The bioavailability of curcumin has been found to improve through its botanical formulation (a complex of curcumin and phosphatidylcholine). The presence of phospholipids in the plant confers its physical and chemical properties including on amphibians, allowing it to disperse in both hydrophilic and lipophilic conditions. Curcumin has been shown to be effective and safe in treating a variety of human disorders, including cancer, arthritis, diabetic microangiopathy, retinopathy, and inflammatory diseases (Mirzaei *et al.* 2017).



Figure 1: Representing the natural plant curcumin.

5. BACTERIA

They are microscopic organisms with different shapes, sticks, spherical, and spiral, that combine with each other and form multiple shapes, such as a rosary or knot, and they are called streptococci, and they are in the form of a conglomerate, and they are called *staphylococcus*. In this study two types of bacteria were used:

5.1. *Escherichia coli*

coli is Gram-negative in diameter ($\sim\mu\text{m}$) and the genome is circular and up to (4.6 Mb). *E. coli* is non-bacterial and has a rod shape and some rods have skin but some are not. This bacteria lives in poultry and livestock. This organism uses simple sugars such as glucose and fermentation to form acetic, formic and lactic acids. *Escherichia coli* is a facultative neurotoxin and strains of *E. coli* caused food poisoning but one case of severe food poisoning was found to be a strain of *E. coli* O157:H7 (Doyle & Schoeni 1984). *Escherichia coli* strains are found in the intestines of humans and animals and have a beneficial function within the human body by collecting quantities of vitamins and preventing harmful bacteria from growing inside the body (Doyle & Schoeni 1984).

5.2. *Staphylococcus aureus*

Staphylococcus is a non-motile Gram-positive spherical bacteria, and this bacteria can grow by fermentation and aerobic respiration (Harris *et al.* 2002) *Staphylococcus aureus* is non-motile and forms grape-like clusters (0.5-1.5 μm in diameter). These organisms constitute facultative anaerobic organisms. Although there are more than 20 species of *Staphylococcus*, only two species of *Staphylococcus*, *S. epidermidis* and *S. aureus*, have been observed to interact with humans. *Staphylococcus aureus* infections are often found in the skin, soft tissues, bones, joints, and internal vascular disorders (Harris *et al.* 2002) *Staphylococcus aureus* is present in the oral cavity and gastrointestinal tract, causing skin infections, and it has been observed that *S. epidermidis* can colonize the skin. Furthermore, *Staphylococcus aureus* is one of the leading causes of foodborne diseases in the world. It causes gastroenteritis with symptoms appearing quickly because it causes food poisoning. (Harris *et al.* 2002).

6. MATERIAL AND METHODS

A colloidal solution of metal nanoparticles was prepared with a high-purity (99.9%) silver target using pulsed laser ablation (PLAL) technology in liquid and at room temperature the metal target was polished and cleaned before and after each excision by washing it with ethanol and then with distilled water using an ultrasound device (ultrasound path) and then cleaning the target to get rid of impurities. The target was placed at the bottom of a glass container and immersed in distilled water (DW). The volume of water used in all excisions was (5 ml) and the height of the liquid above the surface of the target was (4 mm). The energy used is (530 mJ) per pulse. Where the number of laser pulses that bombarded the surface of the metal targets was (600) pulses with a fixed laser energy of (530 megajoules).

In the present work, the distance between the target and the laser lens was (8 cm) and the diameter of the laser beam on the surface of the metal target was (2 mm). The target was bombarded with a laser (Nd: YAG) with a wavelength (1064 nm) with a pulse time (10 ns) and frequency (1 Hz) to obtain the colored colloidal solution containing the removed metallic target nanoparticles as the water changed color after the ablation process

7. RESULTS AND DISCUSSION

1. STRUCTURAL AND MORPHOLOGICAL PROPERTIES OF NANOPARTICLES

X-RAY DIFFRACTION FOR SILVER NANOPARTICLES

The X-ray diffraction results of silver nanoparticles showed that the crystal system is cubic and polycrystalline, and we

notice from Figure (3) the presence of peaks at the angles (38.8), (44.056), (59.732) and that correspond to the levels (111), (200), (105), (220). The obtained results also showed that the distance between the atomic levels (d) and the diffraction angles (2θ) almost corresponding to all diffraction peaks are in good agreement with the standard pattern (JCPDS Card No.02 -1098) and (JCPDS Card No.04-0783) as for the top at the corner (28.434) it corresponds to the level (111) and represents silver oxide according to the standard card number (JCPDS Card No. 40-1054) (Alhamid *et al.* 2019). shows the standard card for silver with the number as shown in the table (1).

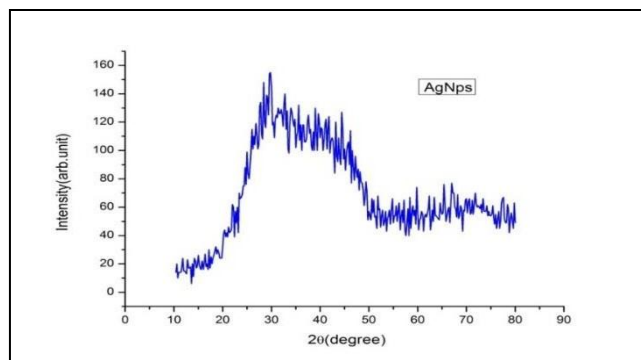


Figure 3 Shows X-ray Diffraction of Silver Nanoparticles

Table 1 Experimental results of X-ray diffraction of silver nanoparticles

2θ (deg) Experimental	FWHM (deg)	D (nm) Crystal size	d_{hkl} (nm) Experimental	Hkl
38.8	0.11252	30.93	0.233	(111)
44.506	0.371	24	0.2034	(200)
59.732	0.528	17	0.15468	(105)
28.434	0.51	16	0.31364	(111)

X-RAY DIFFRACTION FOR SILVER NANOPARTICLES WITH CURCUMIN

Figure (4) shows the (X-ray) pattern of the solution of silver nanoparticles with curcumin, and by analyzing the patterns the locations of the peaks were determined, where we notice the appearance of (104), (200), (111) planes opposite the diffraction angles (50.702), (44.1), (37.9) respectively, which roughly corresponds to the international card (JCPDS Card N0.41-1402) and (03-0921) as shown in Table (2). The information obtained from the X-ray diffraction results showed that all the granules prepared from silver and curcumin are of the cubic type polycrystalline and centered on the face. As for the top at the corner (25.951), which corresponds to the plane (111) it represents silver oxide, according to Card No (40-1054) This is consistent with the researcher's findings (Arunraj *et al.* 2014).

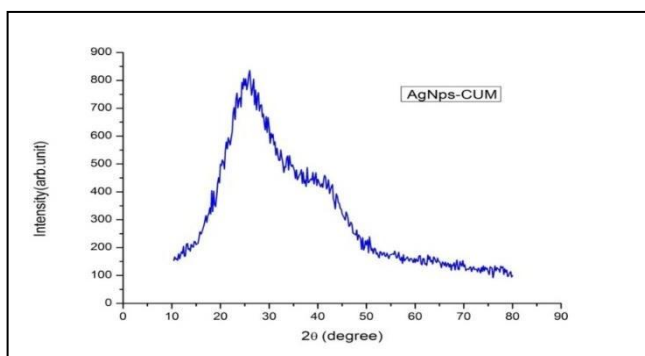


Figure 4 Shows X-ray Diffraction of Silver Nanoparticles with curcmin

Table 2 Experimental results of X-ray diffraction of silver nanoparticle with curcmin

2θ (deg) Experimental	FWHM (deg)	D (nm) Crystal size	d_{hkl} (nm) Experimental	Hkl
37.9	0.95	8.801	2.371	(111)
44.1	0.293	29.31	0.332	(200)
50.702	0.34	27	0.1799	(104)
25.951	0.866	9	0.34306	(100)

THE FIELD EMISSION SCANNING ELECTRON MICROSCOPY(FESEM) RESULTS

Figure (5) shows part a, b (FESEM) images of the diameter distribution of the prepared (AgNps) with (600) (530mJ) where the field emitting electron microscope results showed that the silver nanoparticles semi spherical as well as the appearance of silver particles Large nanoparticles, which were formed due to the gathering of small particles, where Figure (5-a) shows an image of a scale of (200) nm and a magnification of (200 kx) As for the part b of Figure (bb) it represents an image with a scale of (500) nm and a magnification (100) and that the size of nanoparticles varies between(38.32-221.58) nm.

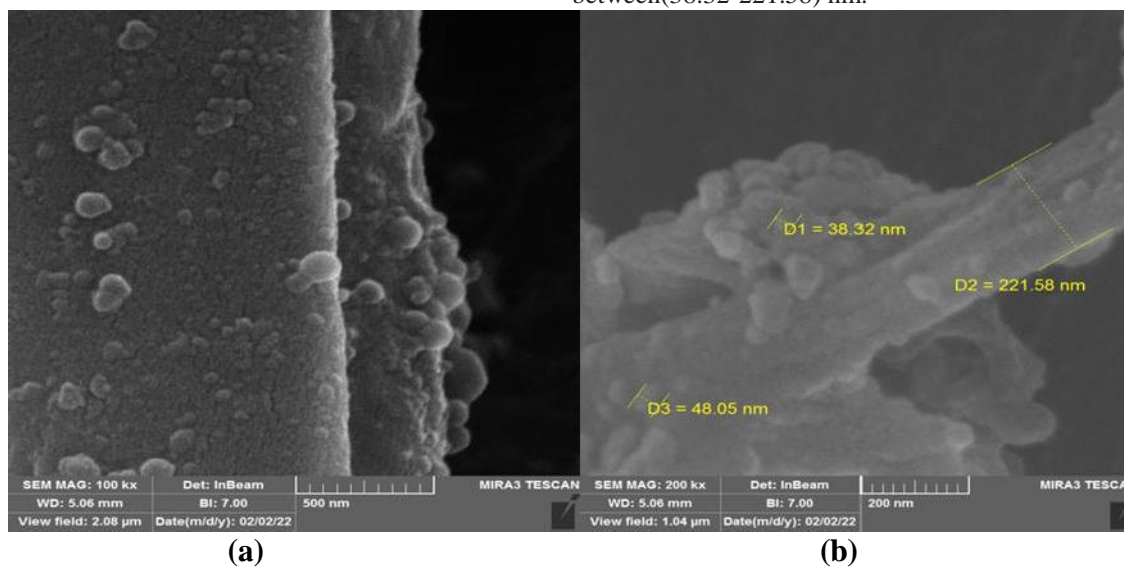


Figure (5) FESEM image of silver nanoparticles

Through the measurement of energy-dispersive X-ray spectrometry (EDX), which is used in order to know the components of the samples, this examination shows the presence of chemical elements in the prepared samples according to their atomic and weight ratios.

Figure (6) shows the (EDX) diagram of the sample (AgNPS) prepared with (600) pulses and ablation energy (600) mJ in a distilled water medium where there are elements (Ag, O, C, Au) some of which are elements present in the surrounding atmosphere (EDX) device, while we notice the appearance of the highest peak of gold (Au) due to coating

the inner surface of the device (EDX) with gold metal. As shown in the table (3).

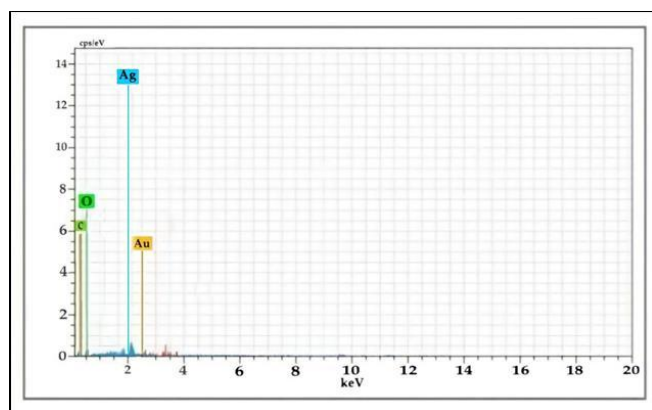
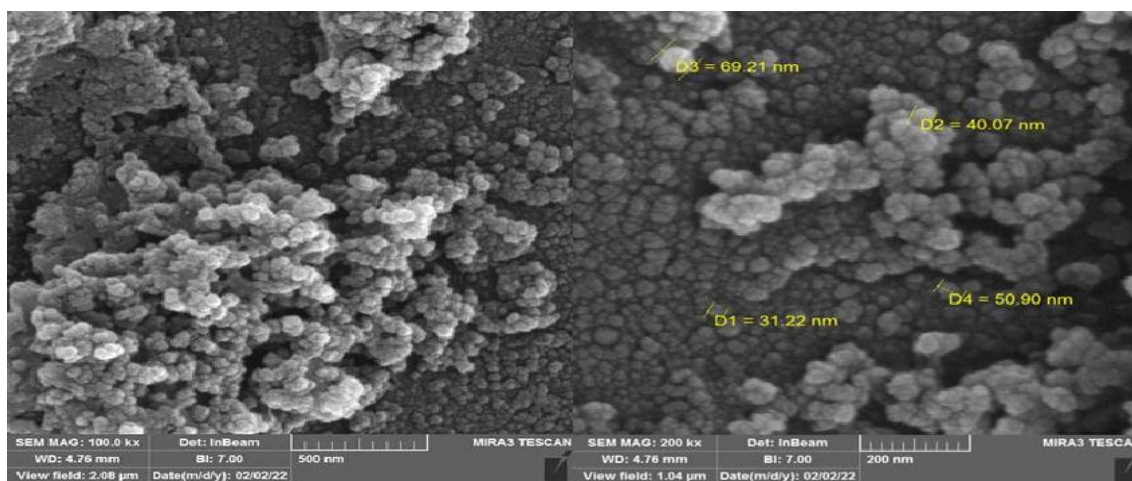


Figure (6) Energy-dispersive X-ray spectrometry (EDX) of the sample(AgNps)

Table 3 Shows the percentages of the elements (AgNps)

Elements	[wt.%]	[at.%]
Ag 47 K-series	62.63	18.45
O 8 K-series	26.29	52.23
C 6 K-series	11.08	29.32
Total	100.00	100.00

Figure (7) shows part a, b (FESEM) images of the diameter distribution of hybrid silver nanoparticles with curcumin prepared with (600) pulses and energy (530 mJ), where the emitting field electron microscope results showed that silver nanoparticles are spherical. As well as the emergence of large silver nanoparticles particles, which were formed as a result of the gathering of small particles, as Figure (7a) shows an image at a scale of (200) nm with a magnification of (200 kx), and part B of Figure (7b) is It represents an image with a scale of (500) nm and a magnification of (100) and the size of nanoparticles ranges between (31.22-69.21) nm.



(a)

(b)

Figure (7) FESEM image of hybrid silver nanoparticles with curcumin

Figure (8) shows the (EDX) diagram of the sample (AgNPS) prepared with (600) pulses and ablation energy (600) mJ in a distilled water medium where there are elements (Ag, O, C, Si, Au) some of which are elements present in the surrounding atmosphere (EDX) device, while we notice the appearance of the highest peak of gold (Au) due to coating the inner surface of the device (EDX) with gold metal. As shown in the table (4).

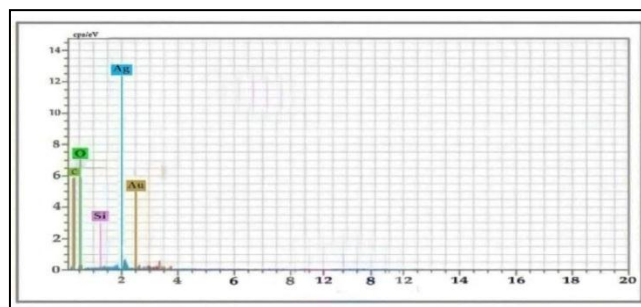


Figure (8) Energy-dispersive X-ray spectrometry (EDX) of the sample (AgNps-Cum)

Table 4 Shows the percentages of the elements (AgNPs)

Elements	[wt.%]	[at.%]
Ag 47 K-series	52.3	48.18
Si 14 K-series	18.8	6.91
O 8 K-series	16.8	6.42
C 6 K-series	12.1	38.49
Total	100.00	100.00

FOURIER TRANSFORM INFRARED TEST RESULTS (FTIR)

One of the most important measurements for examining the bonds between the atomic components of silver solutions is the measurement of Fourier transforms by infrared spectroscopy. Figure (9) shows the relationship between the wave number and transmittance between 500-4000 infrared spectrum of (AgNPs) prepared in in distilled water (DW) with pulse numbers (600) pulses and a constant ablation energy (530 mJ), because it consists of the functional groups of (AgNPs), We see that the nearby wave number's absorption bond (667.27 cm^{-1}) is due to the vibration of the (Ag-O) bond and the absorption bond near the wave number (1045.42 cm^{-1}) is due to the vibration of the stretching (C-O) group which is a group vinyl ether and the absorption bond close to the number (1634.30 cm^{-1}) belong to the group (C=C) which is the conjugated alkene group, and the alkanes group and the adsorption bond ($1771.07\text{-}1732.69\text{-}1843.17\text{ cm}^{-1}$) belong to the group stretching (C=O), which is an vinyl/phenyl ester and aldehyde and vinyl/phenyl ester and straight The absorption bond near wave number (3435.57 cm^{-1}) belongs to the group stretching (OH) which is alcohol As shown in the table (5) (Hamad 2020).

In the figure (10) we note that the absorption bond that appears near the wave number (3451) is a result of the vibrating the (OH) bond back to the alcohol group, and the absorption bond near the wave number (2997 cm^{-1}) is due to the aliphatic (OCH₃(C-H)) group and the absorption bond (2927 cm^{-1}) is the aromatic (C-H) bond and the bond for the wave number (1085 cm^{-1}) is due for the (C-O) group, the bond of absorption at wave number (1045 cm^{-1}) belongs to the (C-OH) bond, and the absorption bond at wave number (1732 cm^{-1}) represents the (C=O) double bond. The absorption bonds near wave number (1651 cm^{-1}) and (1645 cm^{-1}) belong to the aromatic and aliphatic (C=C) group, respectively This confirms that curcumin acts as a capping agent As shown in the table (6) (Verma *et al.* 2016).

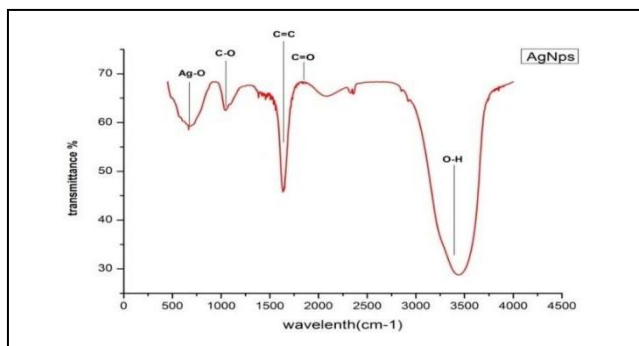


Figure (9) Fourier transforms of the infrared spectrum of a solution of AgNPs

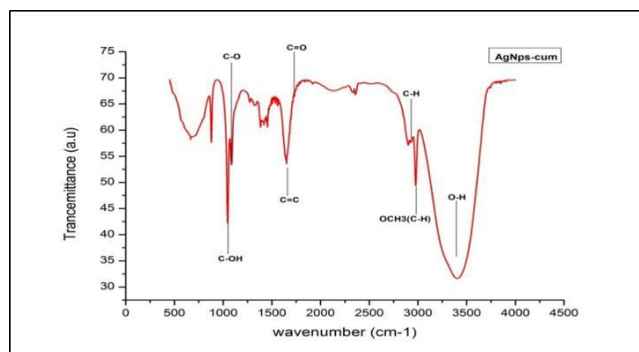


Figure (10) Fourier transforms of the infrared spectrum of a solution of (AgNPs-CUM)

Table 5 FTIR-Spectroscopic data of silver nanoparticles

Functional groups	Absorbance peak (cm^{-1})
Ag-O	667.27 cm^{-1}
C-O	1045.42 cm^{-1}
C = C	1634.30 cm^{-1}
C = O	$(1771.07 - 1732.69 - 1843.17)\text{ cm}^{-1}$
O-H	3435.57 cm^{-1}

Table 6 FTIR-Spectroscopic data of silver nanoparticles with curcumin

Functional groups	Absorbance peak (cm^{-1})
(C=C) the aromatic and aliphatic	$(1645\text{-}1650)\text{ cm}^{-1}$
(C=O)	$(1732)\text{ cm}^{-1}$
(C-OH)	$(1045)\text{ cm}^{-1}$
(C-O)	$(1085)\text{ cm}^{-1}$

aromatic (C-H)	(2927) cm^{-1}
Aliphatic (OCH ₃ (C-H))	(2997) cm^{-1}
OH	(3451) cm^{-1}

2. OPTICAL PROPERTIES

EFFECT OF CURCUMIN CONCENTRATION ON ABSORBANCE AND SURFACE PLASMON RESONANCE

After preparing the colloidal solution containing silver nanoparticles by pulsed laser ablation method at (530) mj energy, (1064) nm wavelength and (600) pulses, the optical properties of silver nanoparticles and curcumin were studied, as well as the study of increasing the concentration of curcumin on the optical properties of the material with a ratio of 1(Ag):1/4 (cum),1(Ag):1/2 (cum), 1(Ag):3/4 (cum) We will notice a change in the shape of the solution with the increase in the concentration of curcumin, as shown in the figure (11) the results of examining the visible rays spectrum showed that it is within the visible spectrum of the electromagnetic rays spectrum within the band (190-110) nm and that the presence of absorption peaks in the visible region is an indication of the formation of nanoparticles, as shown in the figures(12), (13), (14), (15), (16).

In the figure (12) the absorption spectrum shows that the surface plasmon resonance peak site was at the emission spectrum (196 nm) and in the figure (13) demonstrates nanoparticle formation at the spectral emission peak. (190 nm), while in the figure (12) the emission peak was at (202 nm) and in the figure (14) shows the production Nanoparticles at peak spectral emission (210 nm) and in Figure (15) the emission peak is at (216 nm). This shows that increasing the concentration of curcumin was the reason for improving the optical properties of silver nanoparticles and increasing the intensity of surface plasmon resonance and thus increasing the formation of nanoparticles (Abdellah *et al.* 2018)

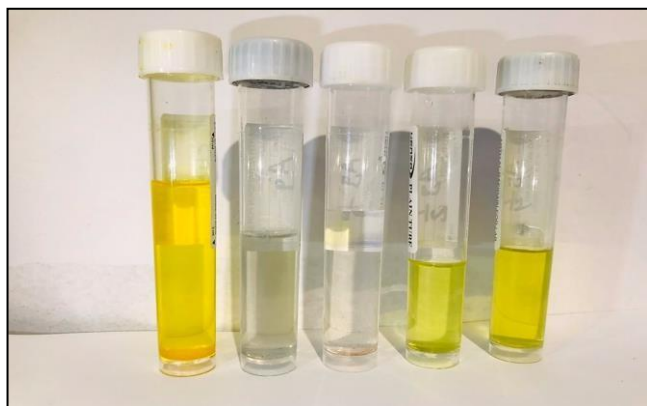


Figure (11) The figure represents the silver nanosilver and curcumin before and after mixing in proportions 1/2 (cum),1/4 (cum),3/4 (cum)

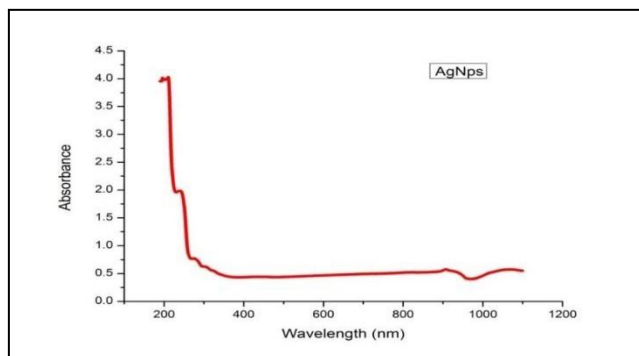


Figure (12) The UV-Visible of silver nanoparticles solution

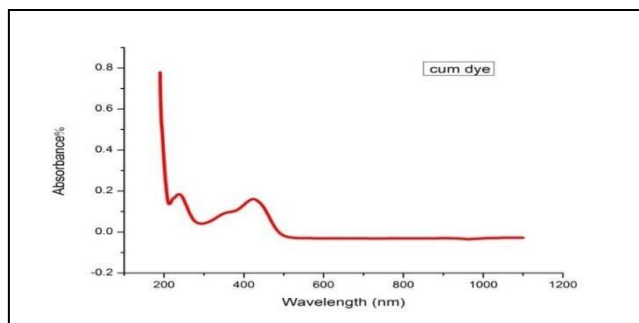


Figure (13) The UV-Visible of curcumin dye

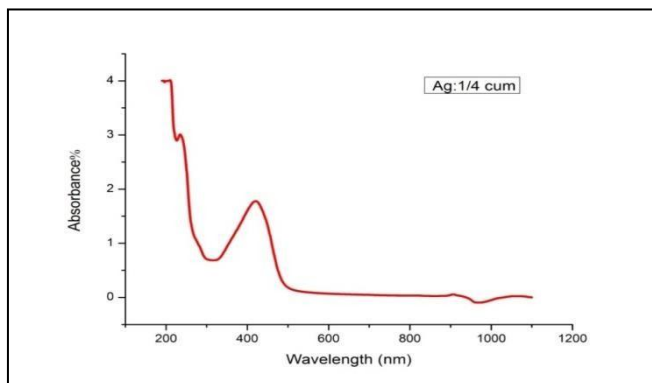


Figure (14) The UV-Visible of hybrid (Ag: cum) at (Ag:1/4 (cum))

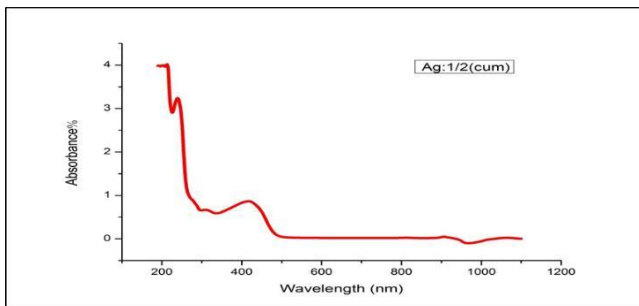


Figure (15) The UV-Visible of hybrid (Ag: cum) at (Ag:1/2 (cum))

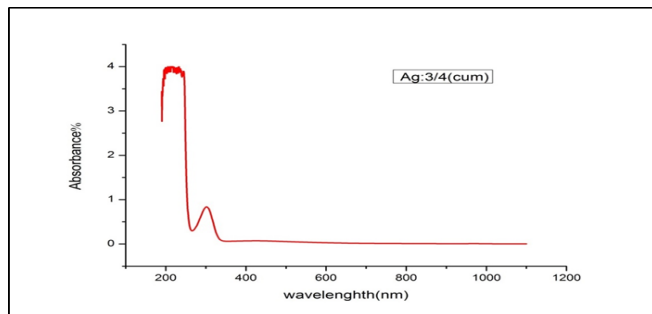


Figure (16) The UV-Visible of hybrid (Ag: cum) at (Ag:3/4 cum)

FLUORESCENCE SPECTRUM RESULTS

Fluorescence is the process of a substance emitting light as a result of absorbing energy, and this energy can be electromagnetic radiation or light. Fluorescence is an emission as a result of the flow of one of the emitted energy inside the illuminated object. The intensity of the fluorescence is directly proportional to the amount of light absorbed by the sample because the fluorescence process depends on this light (Murphy *et al.* 2014). Figure (17) represents the emission spectra of pure silver nanoparticles obtained by the pulsed laser ablation method with a strength of (530) and (600) pulses, we observe that the maximum excitation that is possible was at the wavelength (484 nm).

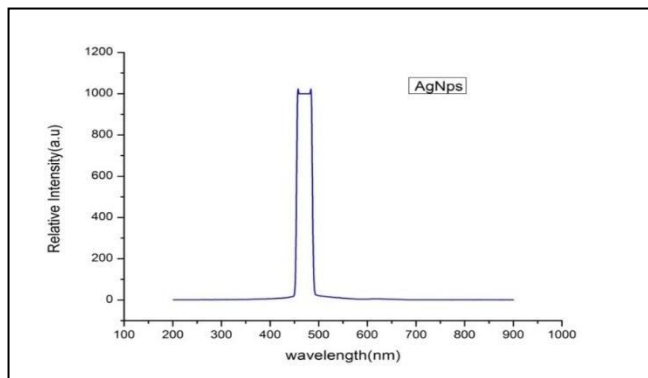


Figure (17) The Fluorescence Spectrum of Silver Nanoparticles

Figure (18) shows the fluorescence measurement of curcumin dye particles, and we see from the graph that the peak value is at (519)

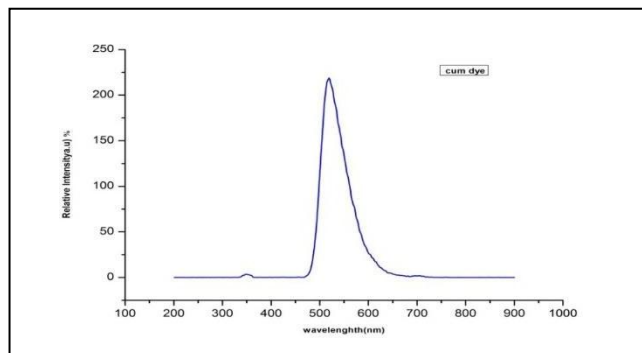


Figure (18) The Fluorescence Spectrum of curcumin dye

Figure (19) the fluorescence measurement is shown of (silver nanoparticles) mixed with curcumin at a ratio of 1:1/4 (cum) and we notice from the graph that the peak value was at the wavelength (295.5 nm) (598 nm) and the highest peak was at the wavelength (598 nm).

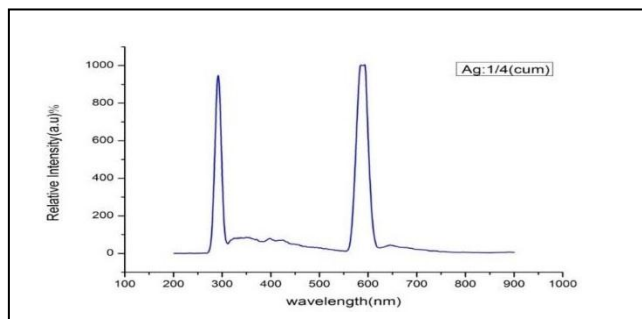


Figure (19) Fluorescence spectrum of silver nanoparticles and curcumin with a ratio (Ag:1/4 cum)

Figure (20) the fluorescence measurement is shown of (silver nanoparticles) mixed with curcumin at ratio 1:1/2 (cum) and we notice from the graph that the peak value was at the wavelength (320.5nm) (650 nm) and the highest peak was at the wavelength (650nm).

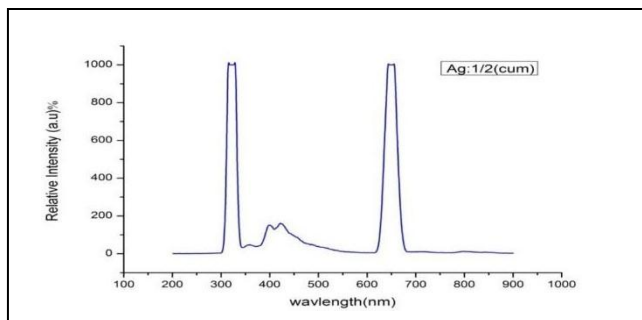


Figure (20) Fluorescence spectrum of silver nanoparticles and curcumin with a ratio (Ag:1/2 cum)

Figure (21) shows the fluorescence measurement of silver nanoparticles mixed with curcumin at a ratio of 1:3/4 (cum) and we notice from the graph that the peak value was at the wavelength (340nm) (660nm) and the highest peak was at the wavelength (660.5nm).

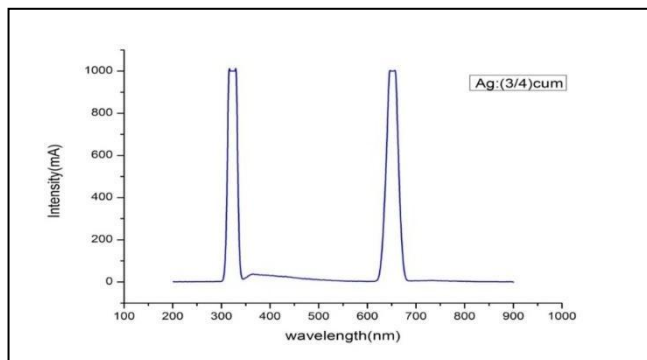


Figure (21) Fluorescence spectrum of silver nanoparticles and curcumin with a ratio (Ag:3/4 (cum))

2 RESULTS OF BIOLOGICAL APPLICATIONS

Inhibitory activity of silver nanoparticles, curcumin and hybrid silver nanoparticles and curcumin was conducted on two types of bacteria (*E. coli*), *Staphylococcus aureus* as shown in the figure (22), (23) Where the results showed that

the hybrid of curcumin with silver particles is more capable of inhibiting bacteria of the mentioned bacterial species, as it showed the ability to inhibit (*E. coli*) with diameter (1.2 mm) and inhibition of (*Staphylococcus aureus*) with diameter (1.4 mm).

The results also showed that curcumin comes in second place after the hybrid of silver and curcumin in the inhibitory activity of the two types of bacteria mentioned, where curcumin showed the ability to inhibit (*E. coli*) in diameter (1.1 mm) and inhibition of (*Staphylococcus aureus*) bacteria in diameter (1.3 mm) The results also showed that silver nanoparticles have the ability to inhibit the mentioned bacterial species in a lower percentage than curcumin, where the silver nanoparticles showed their ability to inhibit (*E. coli*) in diameter (1 mm) and (*Staphylococcus aureus*) bacteria in diameter (1.2 mm) (Abdellah *et al.* 2018). We also note that the bacterial inhibition in the case of distilled water is very small.

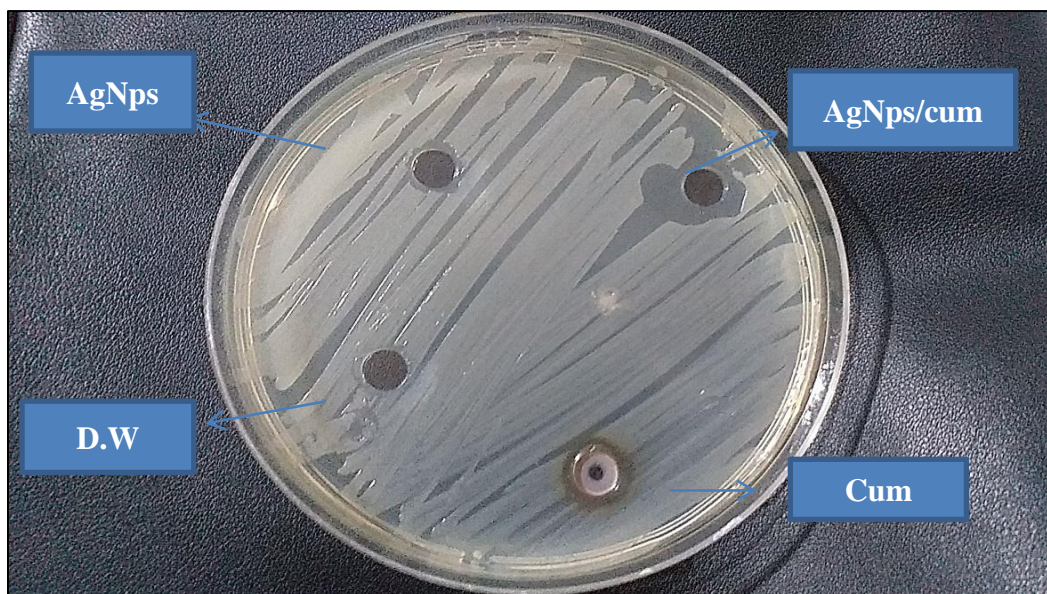


Figure (22) Test the effect of silver nanoparticles, curcumin, and hybrid silver nanoparticles with curcumin and distilled water of bacteria (*E. coli*).

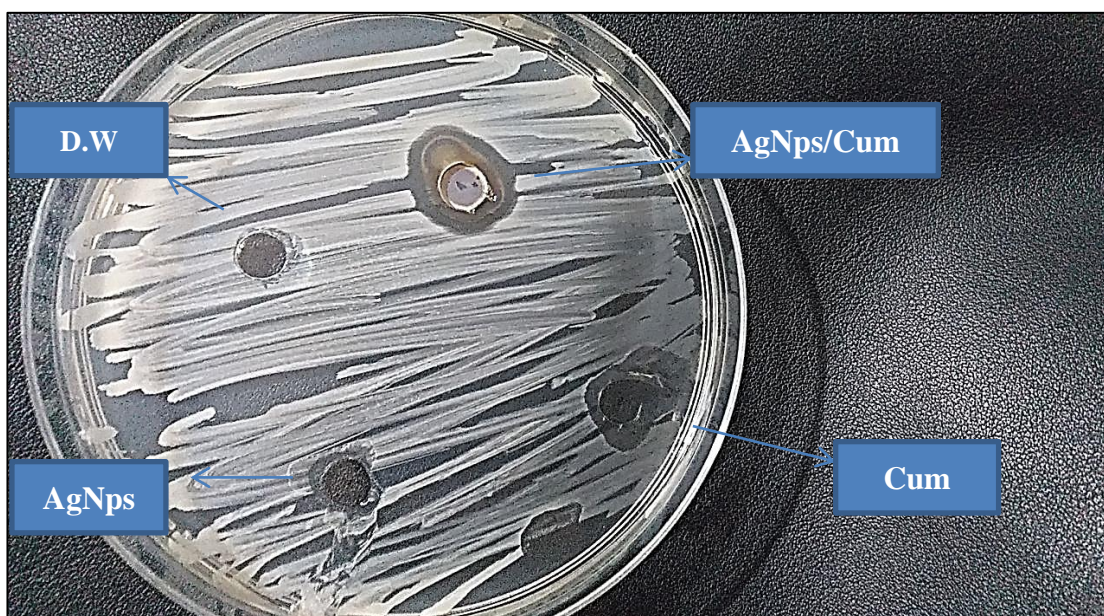


Figure (23) Test the effect of silver nanoparticles, curcumin, and hybrid silver nanoparticles with curcumin and distilled water of (*Staphylococcus aureus*) bacteria

CONCLUSIONS

1. Success of pulsed laser ablation technique in distilled water as a liquid medium for ablation, as the color shift of the solution is an indication of the formation of nanoparticles of the metal used.
2. Studying the optical properties of silver nanoparticles and curcumin and enhancing the optical properties through the hybrid compound.
3. The results of (FESEM) showed that roughly spherical nanoparticles can be obtained.
4. The compound of nanoparticles and hybrids was used in biological applications to inhibit bacterial species, and two types of bacteria were inhibited. The inhibition of the hybrid sample of silver nanoparticles and curcumin was the largest, followed by curcumin and then silver nanoparticles.

RECOMMENDATION

1. Study of ablation with other medium such as deionized water, ethanol, acetone and hydroxide sodium.
2. Synthesis of silver nanoparticles with attach or coating other nanoparticles or other dyes.
3. Using silver nanoparticles and curcumin dye to study another properties and application such as photocatalysis.
4. Using silver nanoparticles and curcumin dye to enhance thermal, electrical and optical properties.

5. Study other parameters of the laser such as wavelength (532nm), frequency (2-6) Hz, pulse time and liquid height above the surface of the material.

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