



Republic of Iraq  
Ministry of Higher Education and Scientific Research  
**Al-Zahrawi University College**  
Department of Pharmacy



# **Improving The Efficacy of Tetracycline in Treating of Resistance Bacteria by Nanotechnology Method**

A major qualifying project report submitted to department of  
Pharmacy – College of Al-Zahrawi University in partial  
fulfillment of the degree of Bachelor in Pharmacy

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((بسم الله الرحمن الرحيم))

﴿ وَ لَقَدْ آتَيْنَا دَاوُودَ وَسُلَيْمَانَ عِلْمًا وَقَالَا الْحَمْدُ لِلَّهِ الَّذِي فَضَّلَنَا عَلَى  
كَثِيرٍ مِّنْ عِبَادِهِ الْمُؤْمِنِينَ ﴾ [النمل : 15]

((صدق الله العلي العظيم))

# Dedication

In the name of Allah, the most merciful most compassionate Allah said (May Allah raise those who have believed and attained knowledge degrees).

We don't start the beginnings we don't reach the end except Allah support.  
We don't achieve our goals only with Almighty Allah.

Praise be to Allah, love, thank and gratitude. Praise be to Allah for the starting and the ending, Praise be to Allah who is the God of the worlds.

To the creature of soul and pen to the creature of dust and wind.

To the man who delivery the divine message and trustworthiness to the people.

To the prophet of mercy and holy light.

To the progeny of messenger Ahlulbeit.

To the My heart's wish and closest to me than my soul.

To our master Mohammed ibn AL Hasan the Last imam

To those who teach us that the life is a struggle which its weapon is the science and knowledge: our Parents.

To our mothers who have pure hearts whom Allah recommend us to take care them carefully and righteously.

To whom share our happiness and moments.

To whom who they feel glad for our success: brothers and friends.

To those whom they love us and guide us and they are behind this success:

The head master of department (Dr. Salam Ajeel) and the supervisor (Prof. Dr. Alaa Al-Daamy) and all dear teachers.

Lovely, we dedicate our humble effort.

## Abstract

**Objective:** The present study aims to improvement efficiency of tetracycline antibiotic by nano method.

**Methods:** A nanohybrid antibiotic Tetracycline-ZnO was prepared using direct ion exchange between antibiotic Tetracycline (TET) and zinc oxide layers (ZnO) . The new nano antibiotic was identified by FT-IR spectroscopy, X-Ray Diffraction (XRD), Atomic Force Microscope (AFM) and scanning electron microscopy (SEM). The antimicrobial activity of the nanohybrid tetracycline was studied against *Staphylococcus hominis* and *Escherichia coli* isolated from patient, that diagnosed by Vitek.

**Results:** The results showed that FT-IR spectroscopy results for the prepared antibiotics showed that the frequencies of some chemical groups shifted towards high and low frequencies. XRD also revealed the emergence of new diffraction planes in the spectrum of the nanohybrid antibiotic compared to the carrier spectrum Zinc oxide, which indicates that the prepared antibiotics under study is nanohybrid antibiotics. Results of atomic force microscopy (AFM) showed that the mean dimensions of the nanoparticles diameters of the TET-ZnO was 194 nanometers. Results of scanning electron microscopy (SEM) was converts irregular shapes of zinc oxide into different geometric shapes interspersed with large spaces when forming the hybrid nanocomposite (TET-ZnO) resulting from the direct interaction of the zinc oxide layers with the tetracycline antibiotic.

The results of the statistical analysis showed that the diameter of inhibition increases significantly ( $P \leq 0.01$ ) in free tetracycline and nanohybrid tetracycline at increase in concentration compared with control. The highest diameters of inhibition zone at concentration 1000 µg/ml were (10.5 and 17.5) mm when we used free tetracycline and nano tetracycline against *Staphylococcus hominis*, respectively. While the highest diameters of inhibition zone at concentration 1000 µg/ml were (12.5 and 19.5) mm when we used free tetracycline and nano tetracycline against *E. coli*, respectively.

**Conclusions:** The success of loading tetracycline on zinc oxide as a carrier of the antibiotic. The activity of the nano-hybrid compound of tetracycline and zinc oxide gave a higher inhibition activity compared to free tetracycline if we take into account the percentage of loading the drug on carrier.

**Keywords:** Resistant bacteria, Tetracycline, Nanotechnology, ZnO.

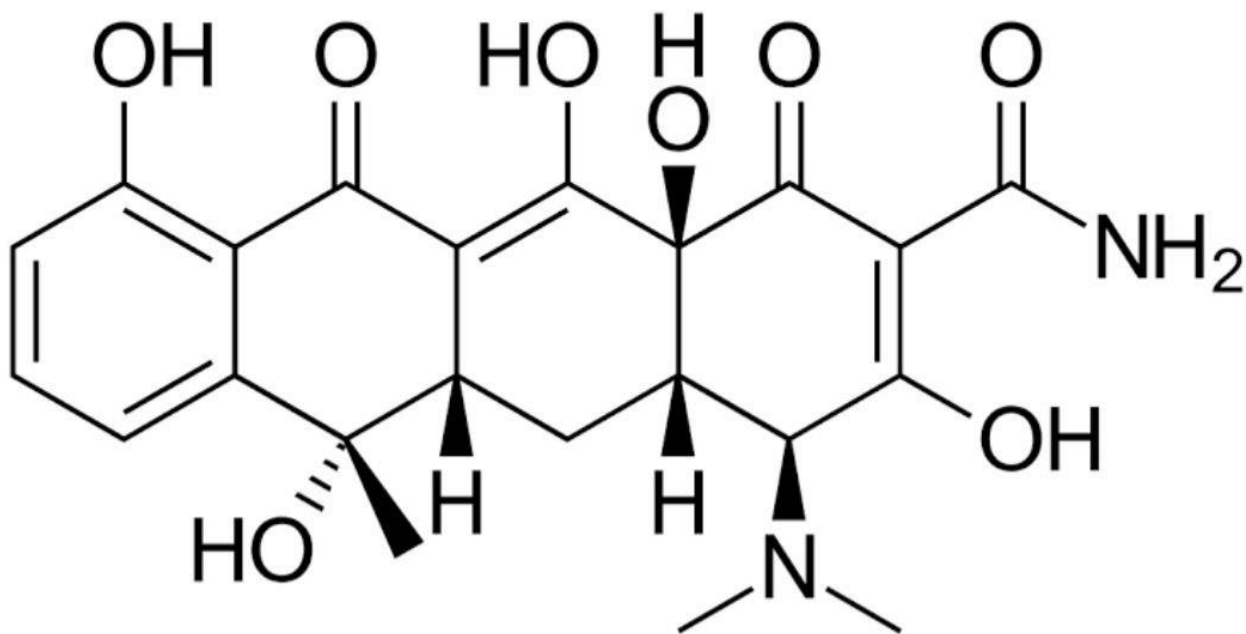
# Introduction

Antibiotics have revolutionized modern medicine, significantly reducing mortality and morbidity associated with bacterial infections. Among the broad spectrum of antibiotics, tetracyclines have played a pivotal role since their discovery, offering effective treatment against various bacterial pathogens. Initially identified as natural products, tetracyclines have evolved over time, leading to the development of synthetic derivatives that have broadened their clinical applications. However, the emergence of antibiotic resistance, particularly against tetracyclines, has posed significant challenges, necessitating innovative approaches like nanotechnology to enhance their efficacy. This paper explores the history, applications, and resistance mechanisms associated with tetracyclines while highlighting the latest advancements aimed at improving their antibacterial properties.

## Discovery and Definition of Tetracycline

Tetracycline was first discovered in the late 1940s as a product of *Streptomyces aureofaciens*, marking a significant breakthrough in antibiotic therapy. Initially known as chlortetracycline, this compound exhibited broad-spectrum activity against a variety of gram-positive and gram-negative bacteria. Subsequently, other tetracyclines were derived through chemical modifications, leading to a family of antibiotics characterized by a four-ring molecular structure. Tetracyclines function by inhibiting bacterial protein synthesis through binding to the 30S ribosomal

subunit, thus preventing the attachment of aminoacyl-tRNA to the ribosome [1].



### Figure 1. Structure of Tetracycline

## Generations of Tetracycline Antibiotics

Tetracyclines are classified into three generations based on their development timeline and structural modifications. First-generation tetracyclines, including tetracycline and chlortetracycline, were derived from natural sources. Second-generation derivatives like doxycycline and minocycline were semi-synthetic, offering improved pharmacokinetic properties such as better absorption and longer half-life. The third-generation tetracyclines, exemplified by tigecycline, are fully synthetic and designed to overcome bacterial resistance mechanisms, making them effective against multi-drug-resistant strains [2].

## **Tetracycline Use Against Gram-Positive and Gram-Negative Bacteria**

Tetracyclines exhibit broad-spectrum antibacterial activity, making them effective against both gram-positive and gram-negative bacteria. They are commonly used to treat infections caused by *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, and *Haemophilus influenzae*. Their ability to penetrate bacterial cell walls and inhibit protein synthesis makes them versatile in managing various infections, from respiratory and urinary tract infections to skin and soft tissue infections [3].

### **Pathogenic Bacteria: *Staphylococcus hominis* and *Escherichia coli***

*Staphylococcus hominis* is a coagulase-negative staphylococcus commonly found on human skin but can cause opportunistic infections in immunocompromised individuals, leading to bloodstream infections and endocarditis. *Escherichia coli*, a gram-negative bacterium, is a frequent cause of urinary tract infections, gastroenteritis, and neonatal meningitis. Tetracyclines are effective in treating infections caused by both pathogens, although resistance can limit their efficacy in certain strains [4].

### **Bacterial Resistance to Antibiotics and Tetracycline**

Antibiotic resistance has become a global health crisis, with bacteria developing mechanisms to evade the effects of drugs like tetracycline. Common resistance mechanisms include efflux pumps that expel the antibiotic from the bacterial cell, ribosomal protection proteins that prevent tetracycline binding, and enzymatic inactivation of the antibiotic. The

widespread use and misuse of tetracyclines in human medicine and agriculture have accelerated the emergence of resistant strains, reducing the effectiveness of these antibiotics [5].

### **Nanotechnology to Enhance Antibiotic Efficiency**

Nanotechnology has emerged as a promising strategy to enhance the efficacy of antibiotics, including tetracyclines. By encapsulating tetracycline in nanoparticles, researchers have improved its stability, bioavailability, and targeted delivery to infection sites. This approach minimizes the required dosage, reduces side effects, and can overcome certain resistance mechanisms. Nanoparticle-based delivery systems, such as liposomes and polymeric nanoparticles, have shown promising results in enhancing the antibacterial activity of tetracycline [6].

### **Research Findings on Free and Nano-Enhanced Tetracycline**

Recent studies have compared the antibacterial efficacy of free tetracycline and its nano-enhanced formulations. Results indicate that nano-encapsulation significantly improves the antibiotic's ability to penetrate bacterial biofilms and achieve sustained drug release, leading to enhanced bacterial inhibition. These findings suggest that nanotechnology can restore the effectiveness of tetracycline against resistant bacterial strains and extend its clinical applications [7].



## Materials and Methods

### Sample Collection:

*Staphylococcus hominis* and *Escherichia coli*; isolated from patients and diagnosed by VITK 2 system, were obtained from the postgraduate laboratory in the Department of Biology/College of Education for Pure Sciences/University of Kerbala.

### Preparation of the hybrid nano tetracycline (Tetracycline Nano hybrid):

The hybrid antibiotic was prepared using the method of Kolekar *et al*, [8] as follows:

**Solution of cephalixin:** 0.5 g of tetracycline was dissolved in 50% ethanol to create this solution, and once the dissolution process was finished, more ethanol was added to get the volume up to 50 ml.

**Solution of zinc oxide (ZnO):** This solution was made by dissolving one gram of zinc oxide in fifty percent ethanol. Once the dissolution process was finished, ethanol was also used to modify the volume to fifty milliliters. Creating the hybrid nanocomposite using cephalixin and zinc oxide layers Gel Sol, an ion exchange technique: In accordance with the previously employed methods credited to Kolekar [8].

### Nutrient agar preparation

1. In an appropriately sized glass beaker with 1000 milliliters of deionized water (DDW), 28 grams of the medium powder are added in accordance with the manufacturer's instructions.

2. To fully dissolve the medium, the mixture is then brought to a boil.
3. After that, the dissolved medium is autoclaved for 15 minutes at a pressure of 15 pounds (121 degrees Celsius) to sterilize it.
4. The flask is taken out and allowed to cool to around 40 degrees when the sterilizing procedure is finished. 45°C.
5. Next, in sterile circumstances, the sterile medium is transferred onto sterile Petri plates.
6. The cast plates can be put in a low-temperature hot air oven once they have solidified. for a few minutes to remove any moisture present on the plates before use.

### **Preparation of the required concentrations and Petri dishes**

1. We prepare 36 plates, 18 of which are for testing the hybrid nano tetracycline antibiotic, and 18 of which are for free tetracycline.
2. Label each plate for the free antibiotic and the hybrid nano-antibiotic according to the following concentrations: (0, 62.5, 125, 250, 500, 1000) µg/ml, with three replicates for each concentration.
3. Make holes in the center of all Petri dishes using a cork borer with a diameter of 6 mm.
4. We prepare 12 test tubes to prepare the required concentrations of the antibiotics mentioned in point 2, including 6 for the free antibiotic and 6 for the nano hybrid antibiotic, as shown in Table 1.

### **Stock solution preparation:**

10 milligrams of each antibiotic were weighed separately and put in different test tubes to create the stock solutions for free tetracycline

and hybrid nano tetracycline. The stock solution, which will be utilized in the next procedures to generate the concentrations employed in this investigation, had a concentration of 1000 µg/ml after 10 ml of distilled water was added.

### **Preparation of free and hybrid nano tetracycline concentrations:**

In accordance with the methodology, the concentrations utilized in this investigation for free tetracycline and tetracycline nano hybrid were made independently according to Table 1.

**Table 1: Preparation of antibiotic concentrates.**

| No. of tube | Distal Water (µl) | Stock Solution (µl) | Final Volume (µl) | Final Concentration (µg/ml) |
|-------------|-------------------|---------------------|-------------------|-----------------------------|
| 1           | 1000              | 0                   | 1000              | 0                           |
| 3           | 938.5             | 62.5                | 1000              | 62.5                        |
| 4           | 875               | 125                 | 1000              | 125                         |
| 5           | 750               | 250                 | 1000              | 250                         |
| 6           | 500               | 500                 | 1000              | 500                         |
| 7           | 0                 | 1000                | 1000              | 1000                        |

### **Characterization of the nanohybrid antibiotic:**

The nanohybrid antibiotic under study was characterized by using several methods including Fourier transform infrared spectroscopy (FT-IR); X-ray diffraction (XRD); Atomic Force Microscope, AFM) and precise analysis of C, H and N elements.

1. **FT-IR:** The infrared spectrum for each of nanohybrid tetracycline and tetracycline free as well as the Zinc oxide (ZnO) was assessed,

by making disk from the compound under study with potassium bromide (KBr) after grinded well, and measuring the infrared spectrum in a wave number range (500-4000)  $\text{cm}^{-1}$ .

2. **X-ray:** diffraction spectrum was used to characterize the nanohybrid tetracycline. XRD explains the difference in the thickness of the layer before and after the intercalation process for tetracycline antibiotic by using Brack's law ( $n\lambda = 2d\sin\theta$ ).
3. **Atomic Force Microscope (AFM):** In order to measure the diameters, sizes and aggregation of the nanoparticles, the samples of the nanohybrid tetracycline were characterized by AFM.
4. **Analysis of CHNS Elements:** The proportions of C, H, N, and S in free and hybrid nanocapsule tetracycline were examined.
5. **Scanning Electron Microscope (SEM):** The exterior surface of tetracycline nanoparticles and free ZnO layers are also examined using a Scanning Electron Microscope (SEM).

### **Measuring the antibacterial efficacy of free and hybrid nano Tetracycline:**

The effectiveness of the free and hybrid nano tetracycline antibiotics was examined using the methodology outlined by El-Rabi [9], as explained below:

#### **Broth with nutrients:**

Following the manufacturer's recommendations, 13 grams of the medium were weighed, dissolved in one liter of distilled water, and sterilized for fifteen minutes to create the nutritional broth. The bacteria

were activated using this media. In the center of Muller Hinton Steel is Muller Hinton Agar. To produce this medium, 38 grams of the medium were weighed, dissolved in one liter of distilled water, and then sterilized for 15 minutes. The antibacterial activity of free and hybrid nano tetracycline against the *Staphylococcus hominis* and *E. coli* was examined using these medium.

### **Bacterial activation:**

*Staphylococcus hominis* and *E. coli* were activated in nutrient broth for one hour before using it to pollinate agricultural crops

### **Measuring antimicrobial efficacy:**

After activating the bacteria, three wells (5 mm in diameter) were made in each plate (Muller Hinton agar), and 80 microliters of antibiotic concentration were added to each well. Then, 50 microliters of the activated bacterial suspension were spread on each plate, and the plates were incubated at 37 degrees Celsius for one day. The diameter of the inhibition zone around the wells was then observed and measured using a ruler.

### **Statistical analysis:**

The results were statistically analyzed using the t-test for comparing two means and the one-way ANOVA test at a significance level of 0.05, using the SPSS program, Version 22 [10].

## Results and Discussion

Characterization of the hybrid nan antibiotic:

**FTIR infrared spectrum:** The FT-IR spectrum of zinc oxide (ZnO) showed small peaks and non-characteristic peaks at 690.54, 868.00, 1616.40, and 3419.90  $\text{cm}^{-1}$ , which are attributed to the vibration of the Zn-O metal bond, as zinc oxide is an inorganic oxide, as shown in Figure 2 [11].

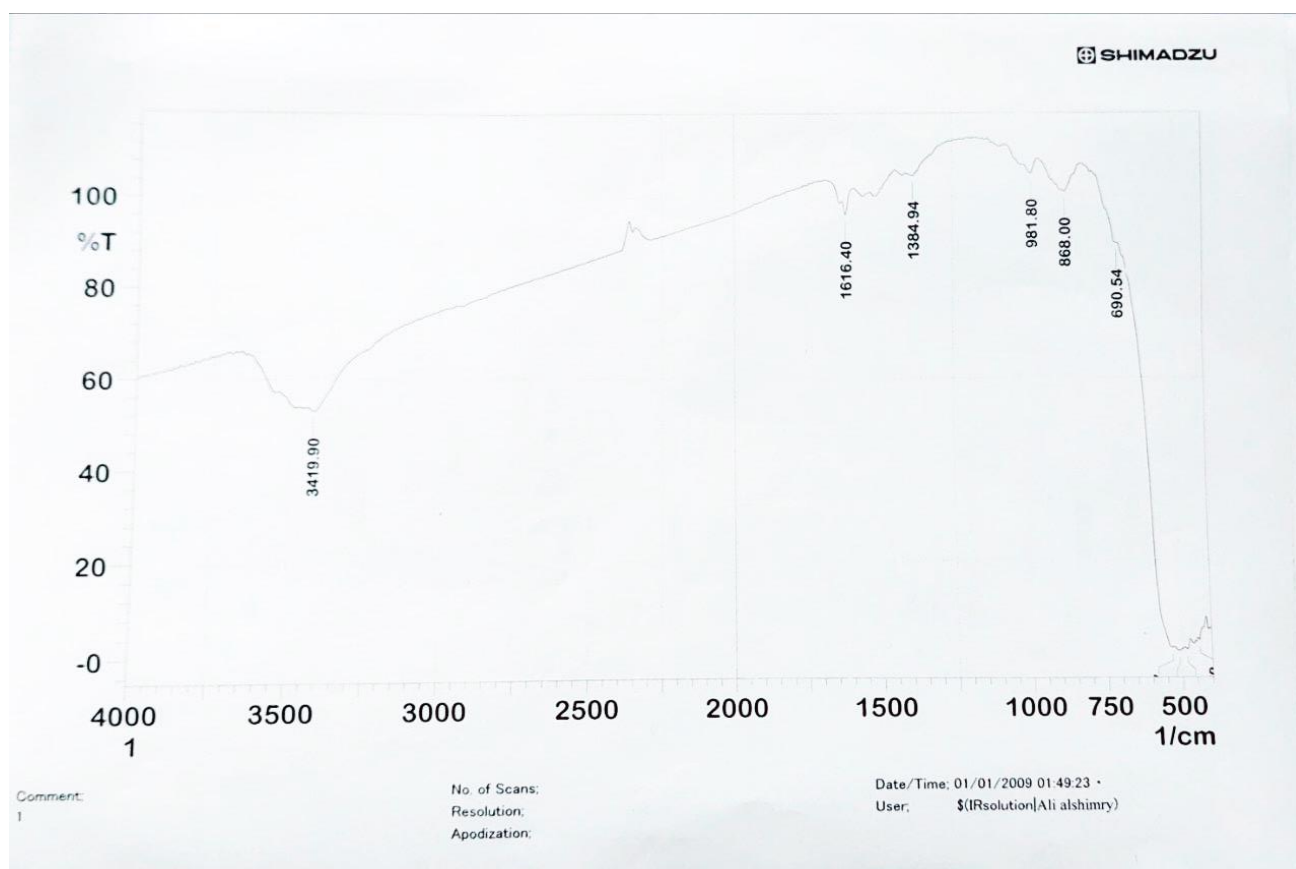
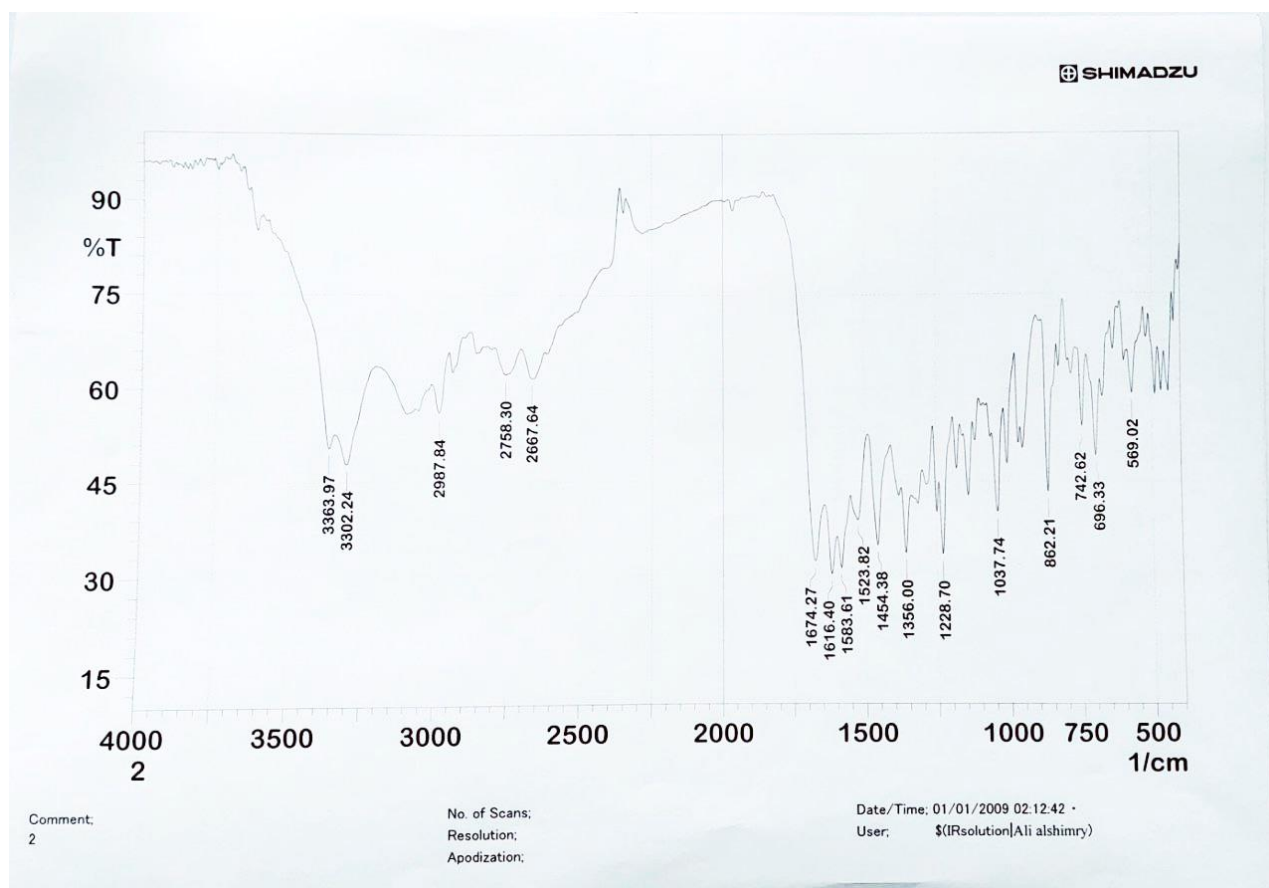


Figure 2. Infrared spectrum (FTIR) for Zinc oxide (ZnO)

The structure of free tetracycline can be confirmed from the FTIR spectrum in Figure 3 and as shown below:

1. Peaks at 3363.97 and 3302.24  $\text{cm}^{-1}$  corresponding to phenolic and alcoholic OH group in addition to  $\text{NH}_2$  group (hydrogen bending).
2. A peak at 2987.84  $\text{cm}^{-1}$  corresponding to CH- aliphatic of  $\text{N}<$  group.

3. A peak at 1674.27 corresponding to  $2C=O$  (ketonic and amidic).
4. Two peaks at 1616.40 and 1583.61  $\text{cm}^{-1}$  corresponding to  $C=C$  bending. In addition to peaks in finger print region from 569.02 to 1523.82  $\text{cm}^{-1}$  [12].



**Figure 3. Infrared spectrum (FTIR) for Tetracycline (TET)**

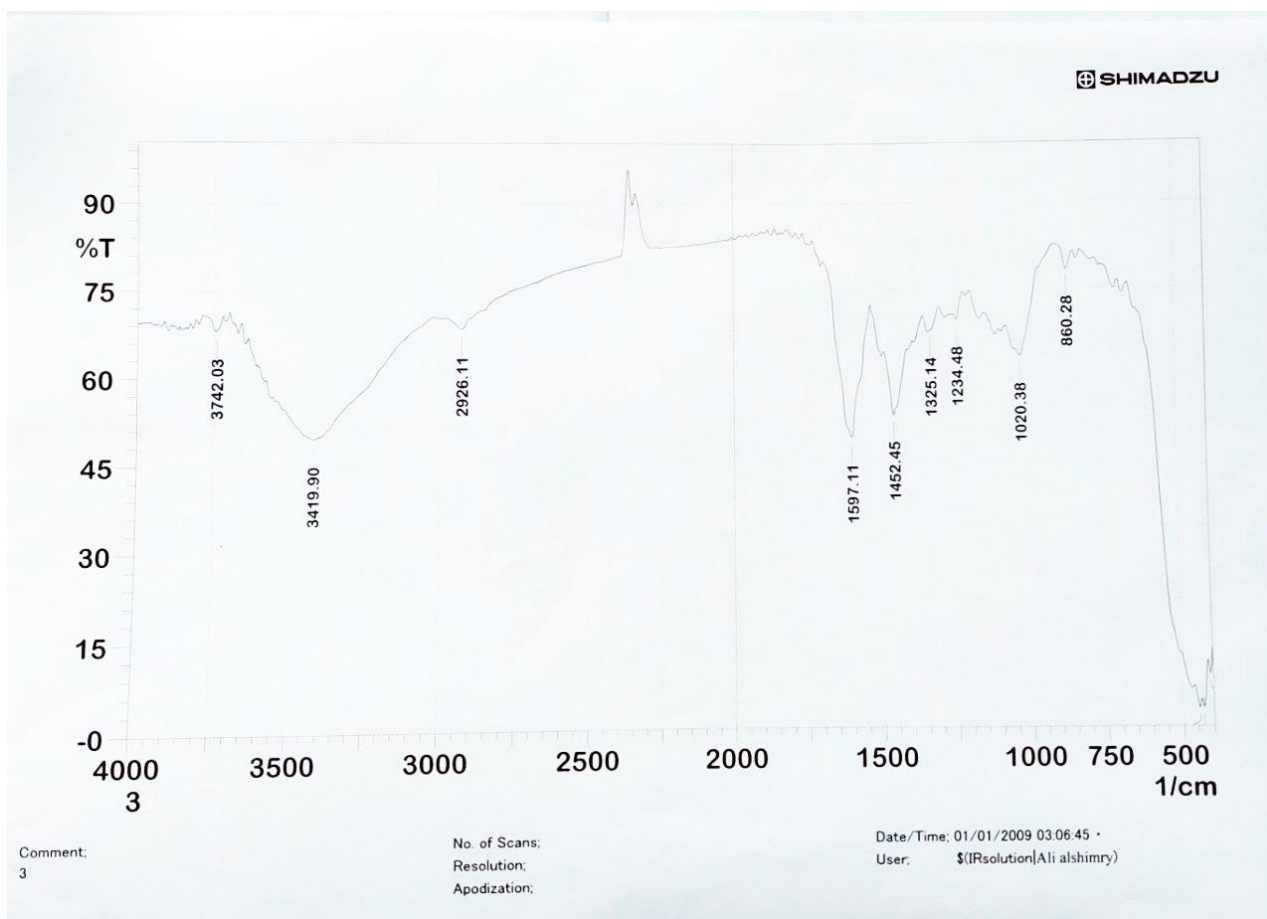
### **Infrared spectrum (FTIR) for nano Tetracycline (nano-TET).**

As shown in **Figure 4** IR spectrum showed of nano-tetracycline-ZnO showed the following absorption peaks:

1. a small peak at 3742.03 ( $\text{NH}_2$  complexed with ZnO) very broad peak at 3419.00  $\text{cm}^{-1}$  ( $\text{OH}$  complexed with ZnO).

2. A small peak at  $2926.11\text{ cm}^{-1}$  corresponding to  $-\text{N}-$ .
3. Disappearance of both  $2\text{C}=\text{O}$  peaks due to complexation with  $\text{ZnO}$ .  

$$>\text{C}=\text{O} \text{ ----- } \text{Zn} \rightarrow >\text{C}-\ddot{\text{O}}-\text{Zn} \text{ [13].}$$

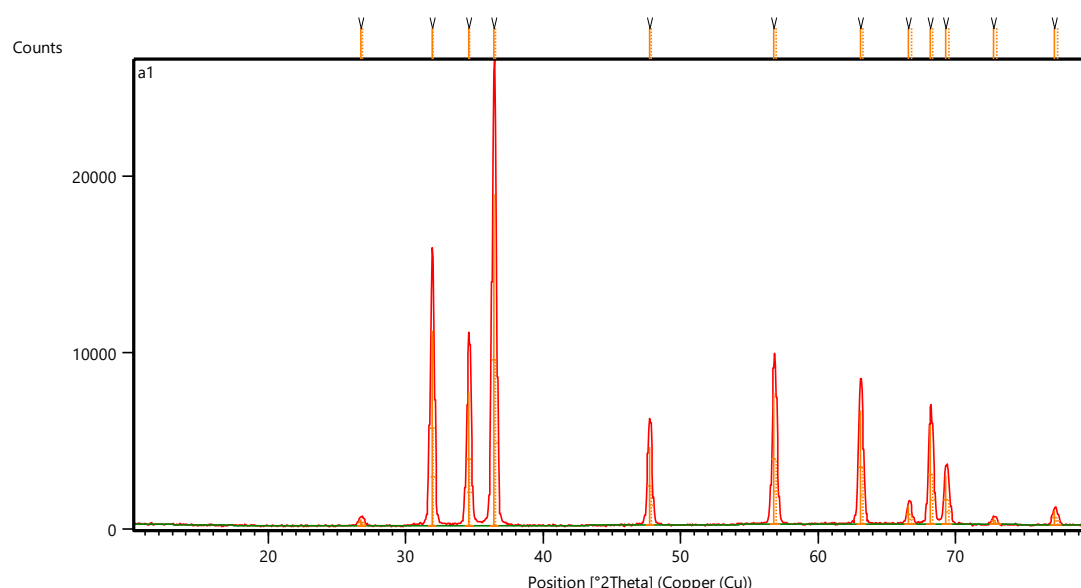


**Figure 4. Infrared spectrum (FTIR) for nano Tetracycline (TET-ZnO)**

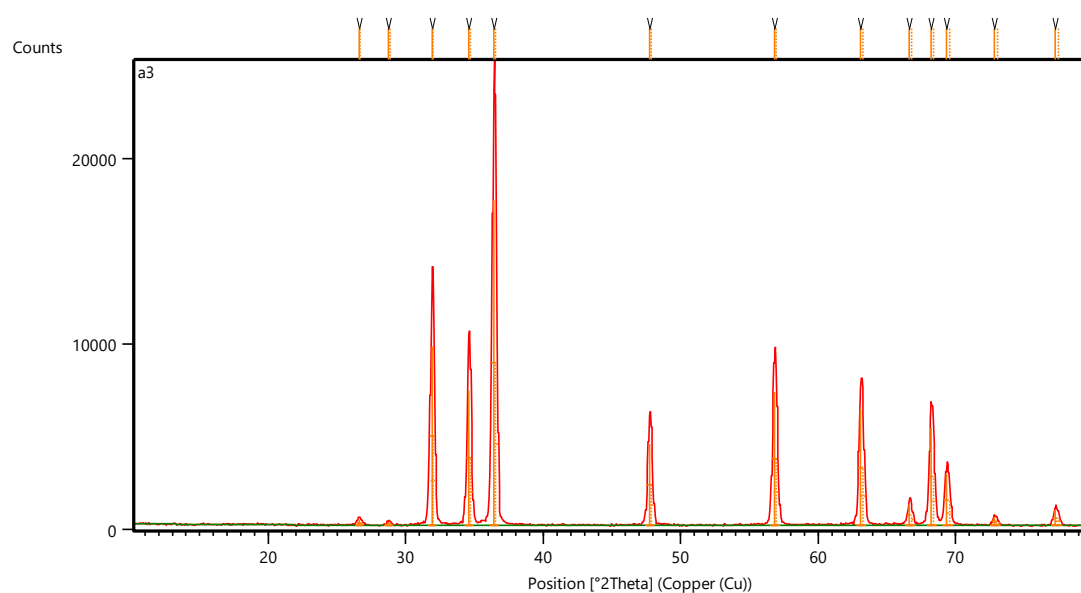
### **Characterization by using X-ray diffraction spectrum (XRD):**

The XRD spectrum of zinc oxide (carrier) and the nanohybrid antibiotic (TET-ZnO) were studied to find the difference in the thickness of the ZnO layers before and after the intercalation of tetracycline between ZnO layers. **Figure 5** illustrated XRD of ZnO while **Figure 6** showed the XRD spectrum of TET-ZnO, results confirmed that tetracycline was intercalated between ZnO layers.





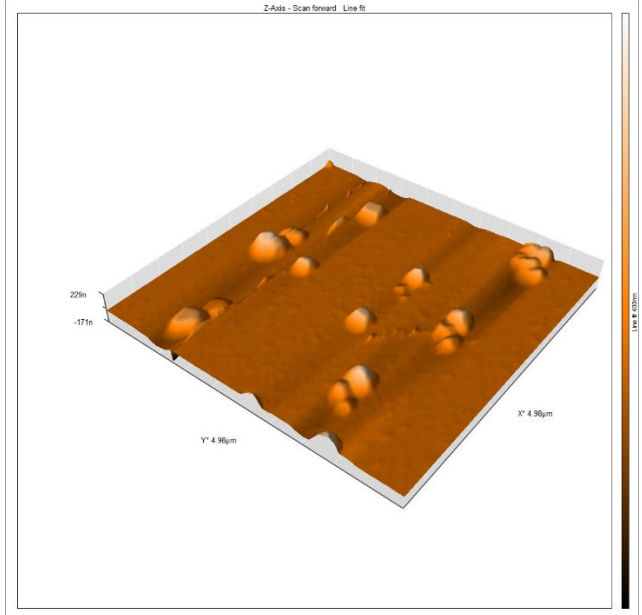
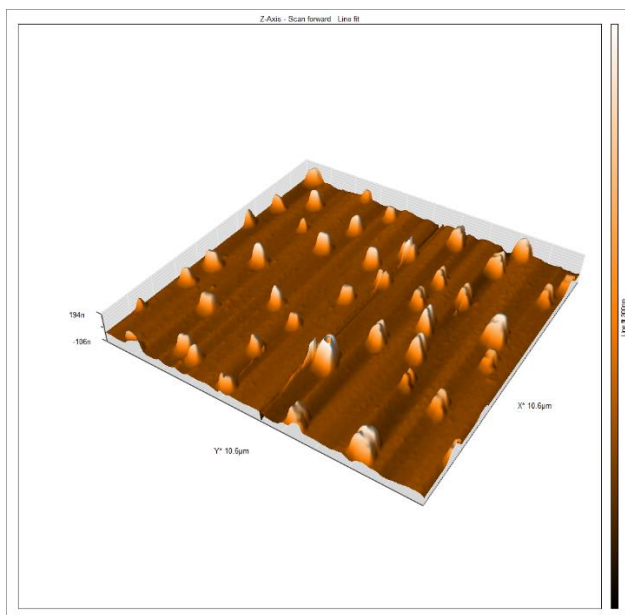
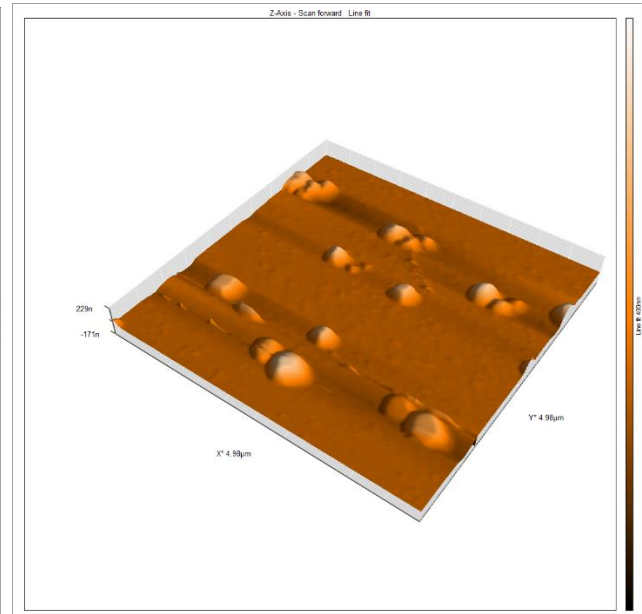
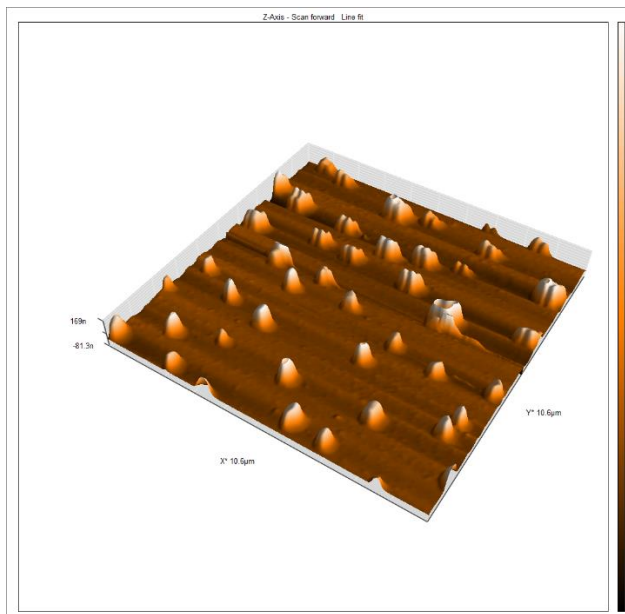
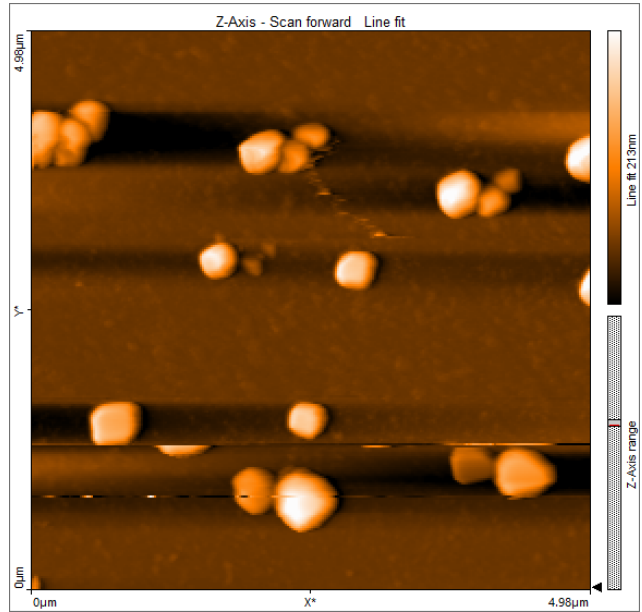
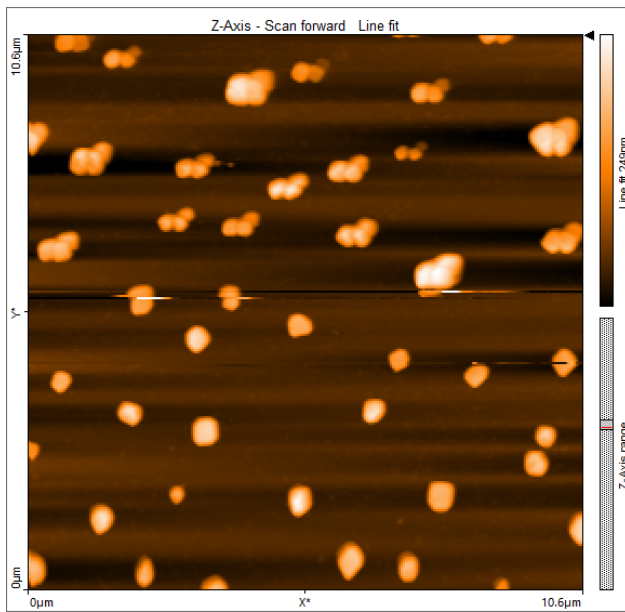
**Figure 5: X-ray diffraction spectroscopy of ZnO.**

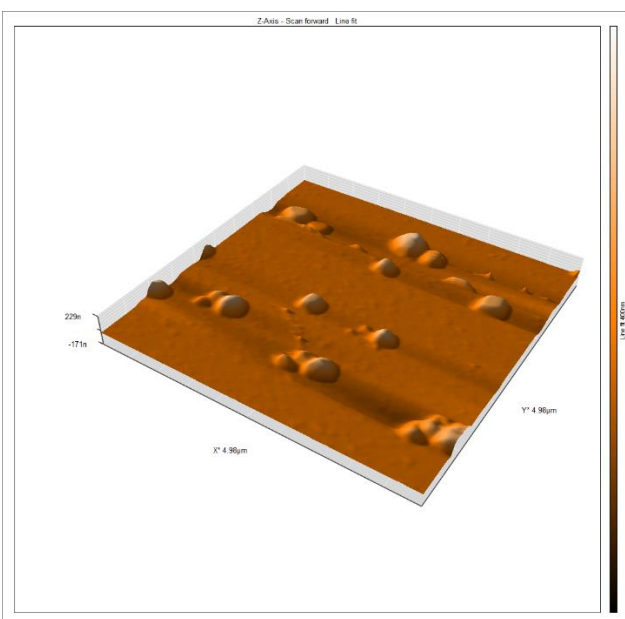
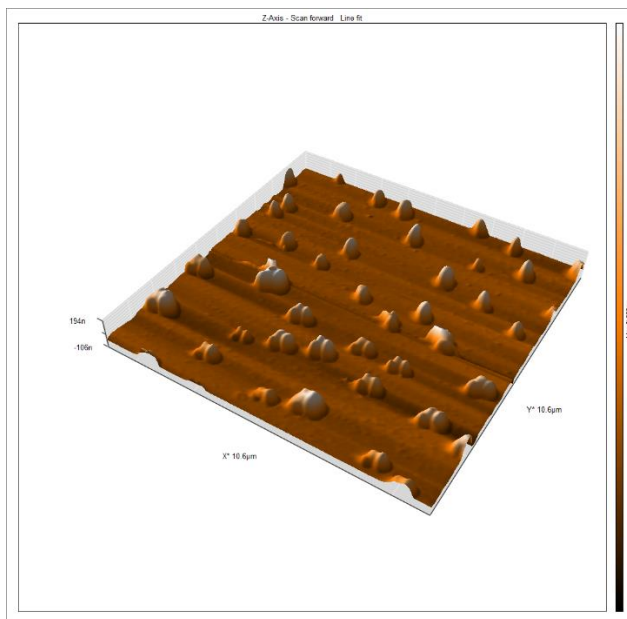


**Figure 6: X-ray diffraction spectroscopy of nano hybrid tetracycline.**

### **Characterization by using Atomic Force Microscope (AFM):**

AFM was used to study the outer surface of the nanohybrid tetracycline TET-ZnO. **Figure 7** showed semispherical forms of TET-ZnO in the two dimensional image. **Figure 7** showed a three dimensional image of the surface section of the nanohybrid antibiotic indicating the successful of preparation of nanohybrid antibiotic where the elevation of molecular assemblies of up to 194 nm.





17/11/2024

## Roughness parameters



### ISO 25178 - Primary surface

S-filter (As): None

F: [Workflow] Surface, form removed (LSP 3)

#### Height parameters

|     |        |    |                         |
|-----|--------|----|-------------------------|
| Sq  | 58.11  | nm | Root-mean-square height |
| Ssk | 0.8880 |    | Skewness                |
| Sku | 4.642  |    | Kurtosis                |
| Sp  | 284.5  | nm | Maximum peak height     |
| Sv  | 150.3  | nm | Maximum pit depth       |
| Sz  | 434.8  | nm | Maximum height          |
| Sa  | 41.32  | nm | Arithmetic mean height  |

#### Functional parameters

|     |       |    |                                |                                  |
|-----|-------|----|--------------------------------|----------------------------------|
| Smr | 100.0 | %  | c = 1000 nm below highest peak | Areal material ratio             |
| Smc | 85.27 | nm | p = 10%                        | Inverse areal material ratio     |
| Sdc | 136.5 | nm | p = 10%, q = 90%               | Material ratio height difference |

#### Spatial parameters

|     |       |    |                      |                             |
|-----|-------|----|----------------------|-----------------------------|
| Sal | 481.1 | nm | s = 0.2              | Autocorrelation length      |
| Str | ****  |    | s = 0.2              | Texture-aspect ratio        |
| Std | 180.0 | °  | Reference angle = 0° | Texture direction           |
| Ssw | 45.14 | nm |                      | Dominant spatial wavelength |

#### Hybrid parameters

|     |        |   |                                  |
|-----|--------|---|----------------------------------|
| Sdq | 0.4802 |   | Root-mean-square gradient        |
| Sdr | 6.020  | % | Developed interfacial area ratio |

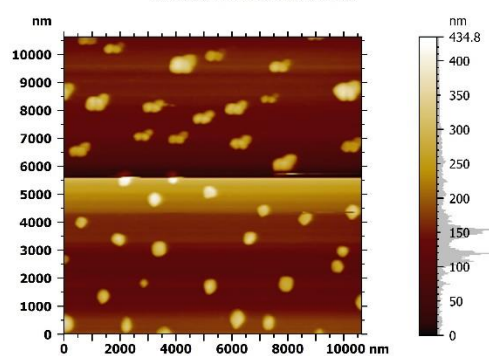
#### Functional parameters (volume)

|     |       |                                  |                  |                      |
|-----|-------|----------------------------------|------------------|----------------------|
| Vm  | 4.186 | nm <sup>3</sup> /nm <sup>2</sup> | p = 10%          | Material volume      |
| Vv  | 89.46 | nm <sup>3</sup> /nm <sup>2</sup> | p = 10%          | Void volume          |
| Vmp | 4.186 | nm <sup>3</sup> /nm <sup>2</sup> | p = 10%          | Peak material volume |
| Vmc | 40.10 | nm <sup>3</sup> /nm <sup>2</sup> | p = 10%, q = 80% | Core material volume |
| Vvc | 84.13 | nm <sup>3</sup> /nm <sup>2</sup> | p = 10%, q = 80% | Core void volume     |
| Vvv | 5.321 | nm <sup>3</sup> /nm <sup>2</sup> | p = 80%          | Pit void volume      |

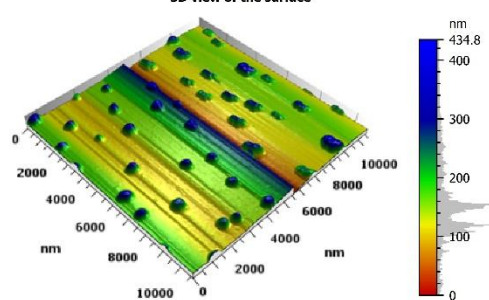
#### Feature parameters

|      |           |                   |              |                                |
|------|-----------|-------------------|--------------|--------------------------------|
| Spd  | 4.072e-07 | 1/nm <sup>2</sup> | Pruning = 5% | Density of peaks               |
| Spc  | 0.01204   | 1/nm              | Pruning = 5% | Arithmetic mean peak curvature |
| S10z | *****     | nm                | Pruning = 5% | Ten point height               |
| S5p  | 255.5     | nm                | Pruning = 5% | Five point peak height         |
| Sdv  | *****     | nm <sup>3</sup>   | Pruning = 5% | Mean daled volume              |

### Pseudo-color view of the surface

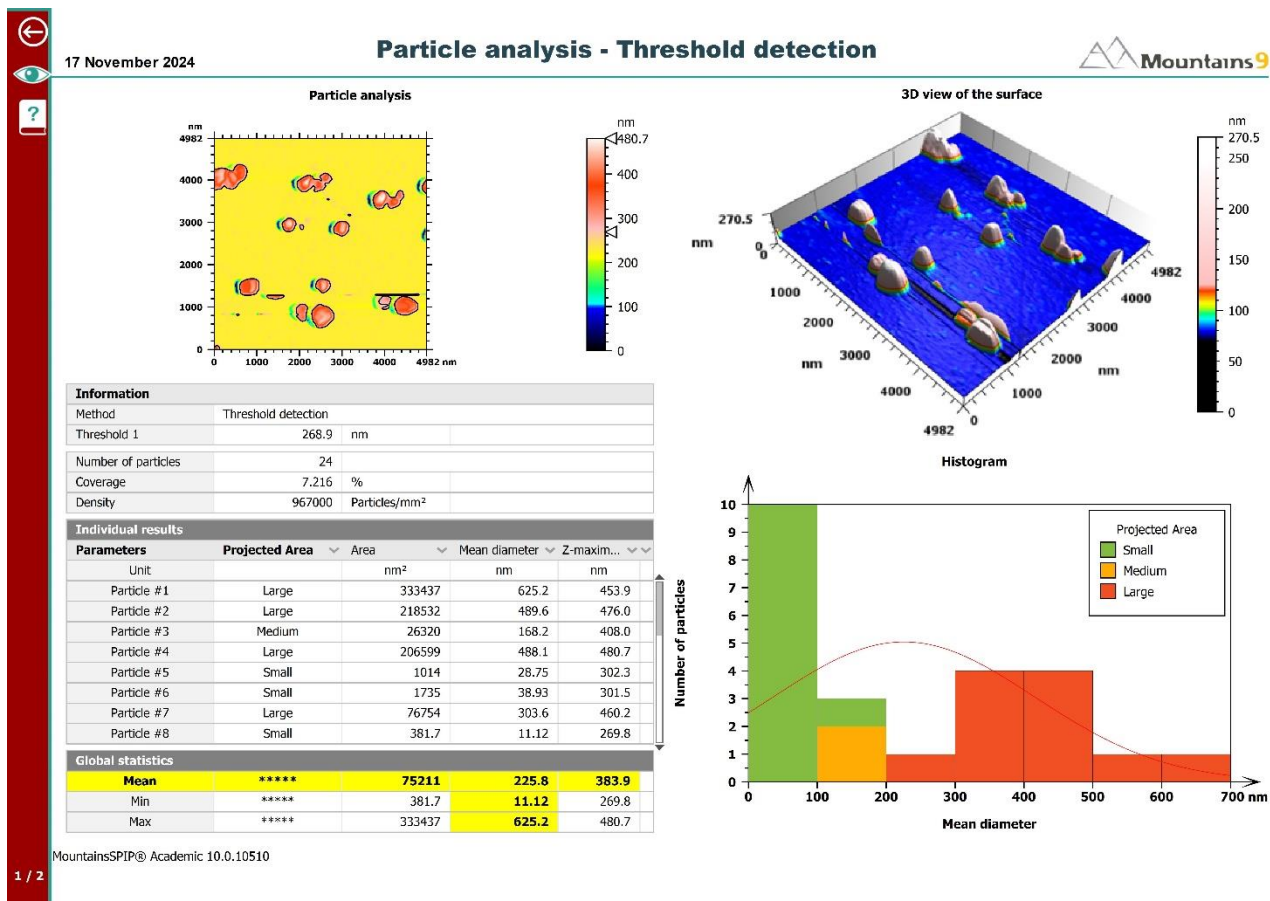


### 3D view of the surface



MountainsSPIP® Academic 10.0.10510

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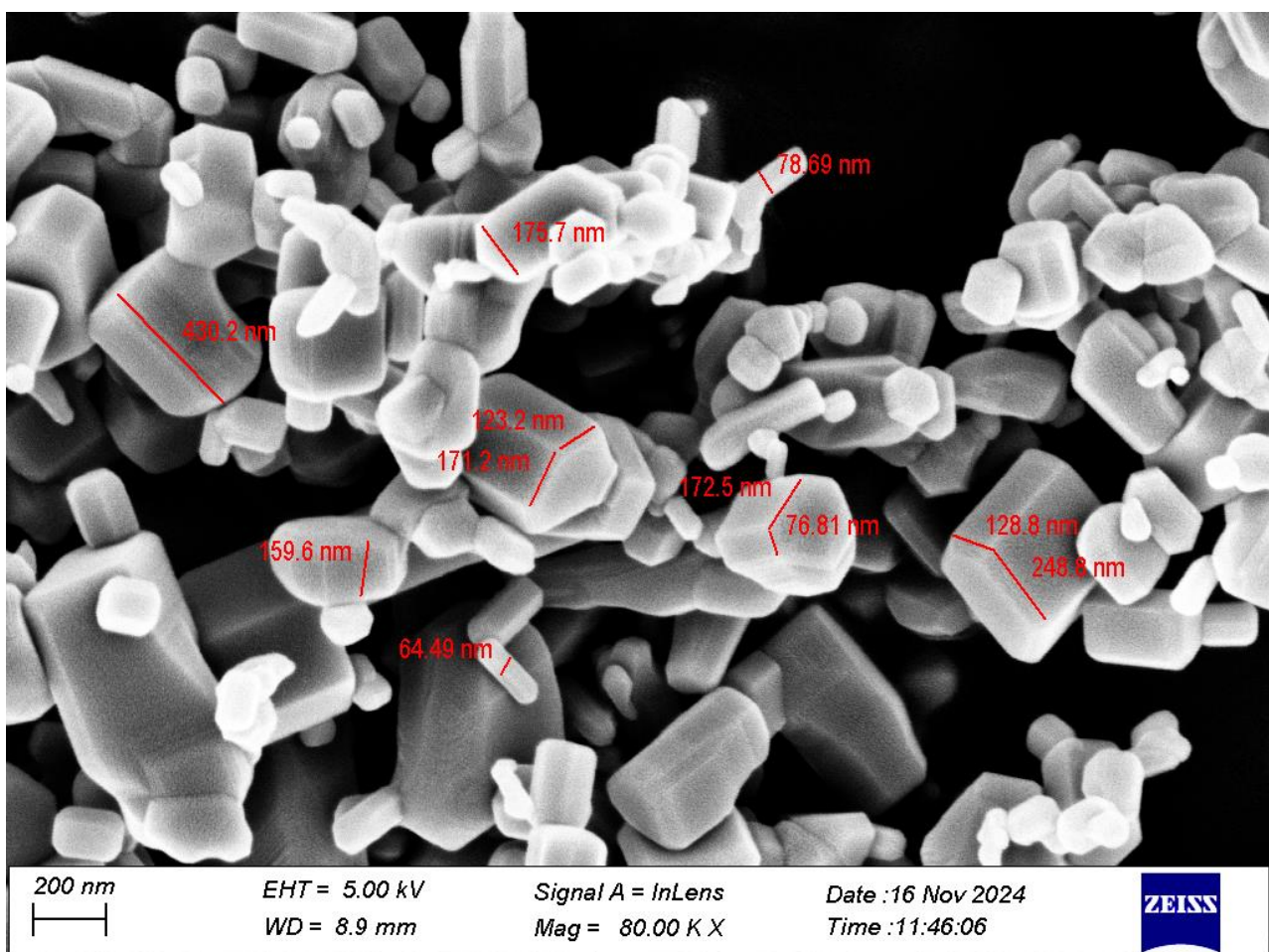
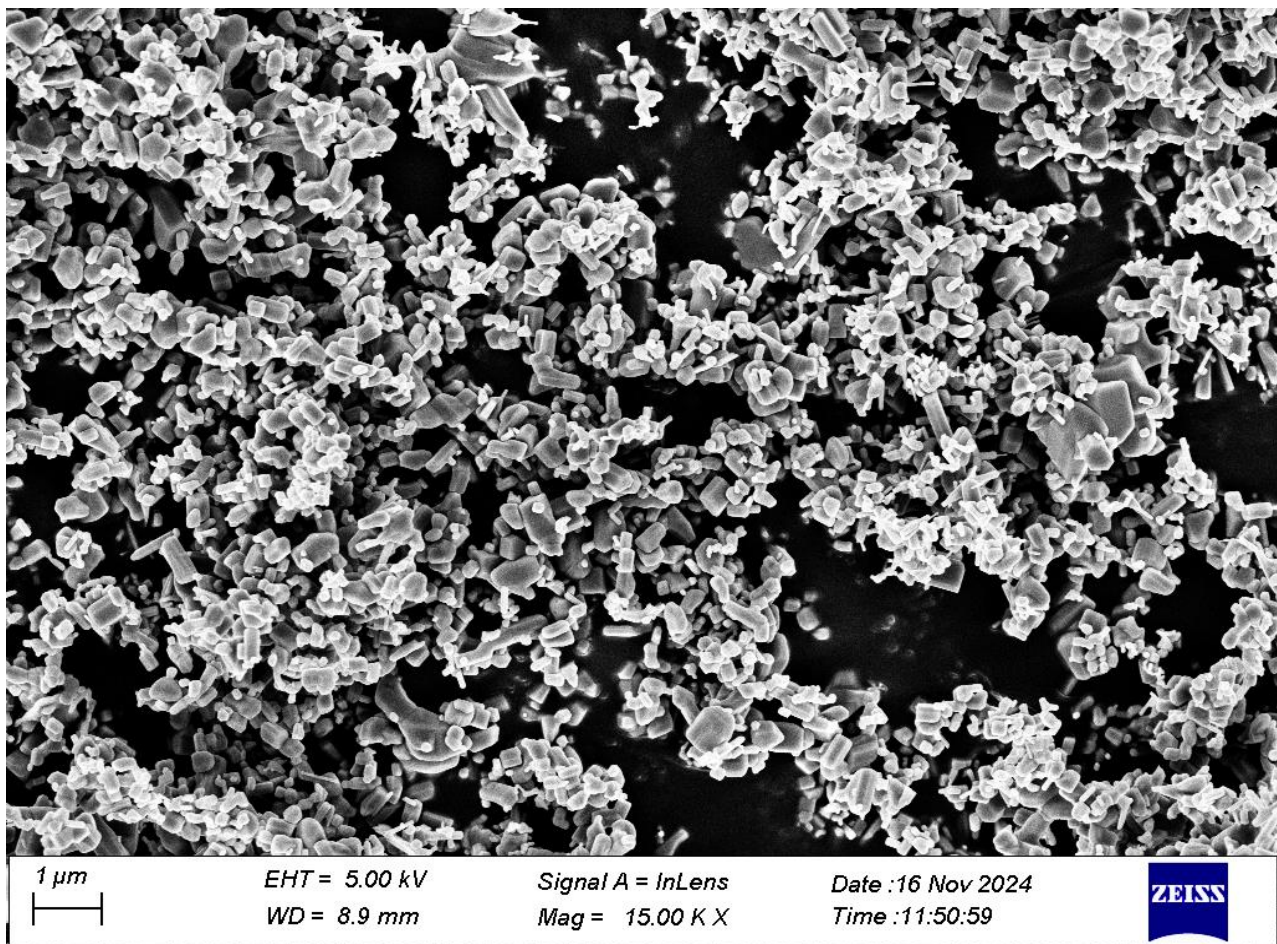


**Fig. 7: Two-dimensional and three-dimensional, image of the TET-ZnO**

### Characterization by using Scanning Electronic Microscope (SEM):

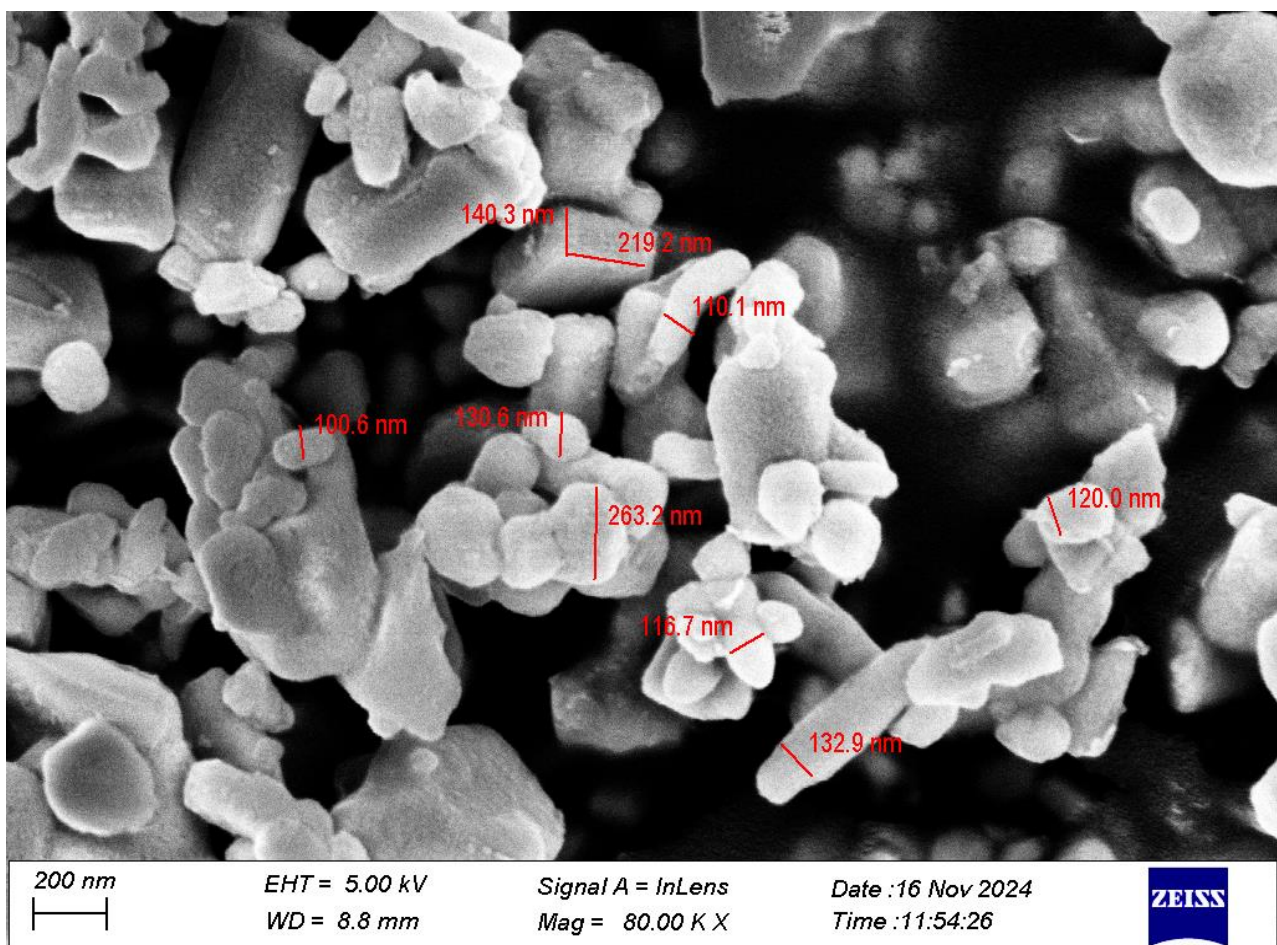
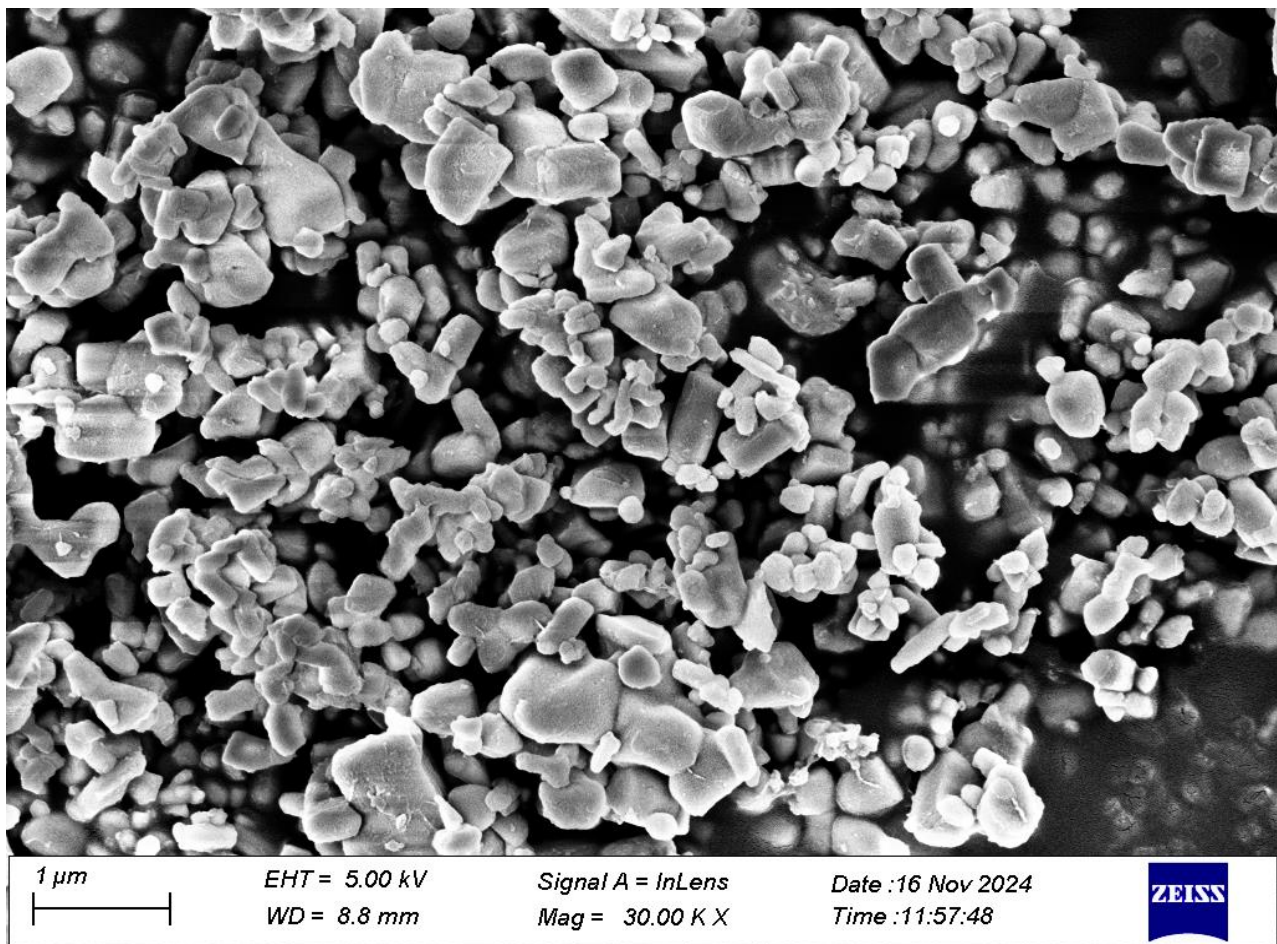
The **Figure 8** shows the scanning electron microscope image of the layers of zinc oxide, where it is noticed that the clear-cut hexagonal shapes in which the oxide leaves appear superimposed on top of each other in irregular shapes and sizes (Bashi *et al*; 2013) [14], and that these irregular shapes convert into different geometric shapes interspersed with spaces when the hybrid nanocomposite (TET-ZnO) is formed resulting from the direct interaction of the zinc oxide layers with the tetracycline antibiotic **Figure 9**, which indicates the success of the process of intercalation of the antibiotic into the zinc oxide layers, which is supported by the X-ray diffraction spectrum as It is shown in the **Figure 6**.





**Fig 8: Scanning Electron Microscope (SEM) image of Zinc Oxide layers.**





**Fig 9: Scanning Electron Microscope (SEM) image of Zinc Oxide layers / with TET-ZnO.**

## Precise Analysis of Elements in the Nanohybrid Antibiotic

Elements chemical analysis showed that the percentages of carbon, hydrogen and nitrogen were 54.35, 5.26 and 5.49 % for TET-free, while they were 52.13, 3.57 and 17.52 % for TET-ZnO. These results indicate that the level of tetracycline loaded between the zinc oxide layers was 95.91 % (Table 2 and 3).

Table 2: The components of free Tetracycline

Date : 2024/12/07 at 12:02:43

Method Name : Nitrogen/Carbon/Hydrogen/Sulphur

Method Filename : N C H S system.mth

| Filename       |       | AS Method   |      | Vial  |       |     |
|----------------|-------|-------------|------|-------|-------|-----|
| -----          |       |             |      |       |       |     |
| S0309022       |       |             |      |       |       |     |
| #              | Group | Sample Name | Type | Weig. | Pro.F | --- |
| -----          |       |             |      |       |       |     |
| 164            | 1     | 03.09.022   | UNK  | 2.393 | 6.25  | --- |
| Component name |       | Element %   |      |       |       |     |
| -----          |       |             |      |       |       |     |
| Nitrogen%      |       | 5.492785454 |      |       |       |     |
| Carbon%        |       | 54.35391617 |      |       |       |     |
| Hydrogen%      |       | 5.264844894 |      |       |       |     |
| Sulphur%       |       | 0           |      |       |       |     |

1 Sample(s) in Group No : 1

| Component Name | Average     | Std. Dev. | % Rel. S. D. | Variance |
|----------------|-------------|-----------|--------------|----------|
| -----          |             |           |              |          |
| Nitrogen%      | 5.492785454 | 0         | 0.0000       | 0.0000   |
| Carbon%        | 54.35391617 | 0         | 0.0000       | 0.0000   |
| Hydrogen%      | 5.264844894 | 0         | 0.0000       | 0.0000   |
| Sulphur%       | 0           | 0         | 0.0000       | 0.0000   |

Table 3: **The components of nano Tetracycline**

Date : 2024/12/07 at 12:03:10

Method Name : Nitrogen/Carbon/Hydrogen/Sulphur

Method Filename : N C H S system.mth

| Filename                    |             | AS Method   |           | Vial         |       |          |
|-----------------------------|-------------|-------------|-----------|--------------|-------|----------|
| -----                       |             |             |           |              |       |          |
| S0309024                    |             |             |           |              |       |          |
| #                           | Group       | Sample Name | Type      | Weig.        | Pro.F | ---      |
| -----                       |             |             |           |              |       |          |
| 166                         | 3           | 03.09.024   | UNK       | 2.999        | 6.25  | ---      |
| Component name              |             | Element %   |           |              |       |          |
| -----                       |             |             |           |              |       |          |
| Nitrogen%                   |             | 17.92955017 |           |              |       |          |
| Carbon%                     |             | 52.13680267 |           |              |       |          |
| Hydrogen%                   |             | 3.572915554 |           |              |       |          |
| Sulphur%                    |             | 0           |           |              |       |          |
| -----                       |             |             |           |              |       |          |
| 1 Sample(s) in Group No : 3 |             |             |           |              |       |          |
| Component Name              | Average     |             | Std. Dev. | % Rel. S. D. |       | Variance |
| -----                       |             |             |           |              |       |          |
| Nitrogen%                   | 17.92955017 |             | 0         | 0.0000       |       | 0.0000   |
| Carbon%                     | 52.13680267 |             | 0         | 0.0000       |       | 0.0000   |
| Hydrogen%                   | 3.572915554 |             | 0         | 0.0000       |       | 0.0000   |
| Sulphur%                    | 0           |             | 0         | 0.0000       |       | 0.0000   |

## Antimicrobial Activity of Tetracycline

The results of the statistical analysis in **Table 4** showed that there are a high significant differences ( $P \leq 0.01$ ) in the diameters of inhibition zone of the free tetracycline against *Staphylococcus hominis* at all concentrations that used compared with the control. In addition, there are increasing in inhibition zone when the concentration was increased. The diameters of inhibition zone to Free-TET were (0.5, 2.5, 7.0, 8.0 and 10.5) mm of the following concentrations (62.5, 125, 250, 500 and 1000)  $\mu\text{g/ml}$ ; respectively.

When we used the Nano-TET there are a high significant differences ( $P \leq 0.01$ ) in the diameters of inhibition zone of the nano tetracycline against *Staphylococcus hominis* at all concentrations that used compared with the control. In addition, there are increasing in inhibition zone when the concentration was increased. The diameters of inhibition zone to Nano-TET were (2.5, 5.5, 10.5, 14.0 and 17.5) mm of the following concentrations (62.5, 125, 250, 500 and 1000)  $\mu\text{g/ml}$ ; respectively.



In other hand, when we compare between Free-TET and Nano-TET to each concentration, the results refer to significance differences ( $P \leq 0.01$ ) in all concentrations, separately.

Table 4: The inhibitory efficacy of Tetracycline against *Staphylococcus homonis*

| Concentration<br>( $\mu\text{g/ml}$ ) | Diameter of inhibition zone (mm) |                     | P value  |
|---------------------------------------|----------------------------------|---------------------|----------|
|                                       | Tetracycline (Free)              | Tetracycline (Nano) |          |
| 0 (Control)                           | $0 \pm 0.00$                     | $0 \pm 0.00$        | 1.000    |
| 62.5 ( $\mu\text{g/ml}$ )             | $0.5 \pm 0.07$                   | $2.5 \pm 0.07$      | 0.0001 * |
| 125 ( $\mu\text{g/ml}$ )              | $2.5 \pm 0.121$                  | $5.5 \pm 0.707$     | 0.0019 * |
| 250 ( $\mu\text{g/ml}$ )              | $7.0 \pm 0.07$                   | $10.5 \pm 1.121$    | 0.0057 * |
| 500 ( $\mu\text{g/ml}$ )              | $8.0 \pm 0.07$                   | $14.0 \pm 0.01$     | 0.0001 * |
| 1000 ( $\mu\text{g/ml}$ )             | $10.5 \pm 1.121$                 | $17.5 \pm 0.07$     | 0.0004 * |
| P value                               | 0.0000 *                         | 0.0000 *            |          |
| LSD                                   | 1.815                            | 2.916               |          |

The numbers refer to mean  $\pm$  Standard Deviation

\* refers to high significance differences ( $P \leq 0.01$ )

The results of the statistical analysis in Table 5 showed that there are a high significant differences ( $P \leq 0.01$ ) in the diameters of inhibition zone of the free tetracycline against *E. coli* at all concentrations that used compared with the control. In addition, there are increasing in inhibition zone when the concentration was increased. The diameters of inhibition zone to Free-TET were (0.5, 1.5, 5.5, 10.5 and 12.5) mm of the following concentrations (62.5, 125, 250, 500 and 1000)  $\mu\text{g/ml}$ ; respectively.

When we used the Nano-TET there are a high significant differences ( $P \leq 0.01$ ) in the diameters of inhibition zone of the nano tetracycline against *E. coli* at all concentrations that used compared with the control. In addition, there are increasing in inhibition zone when the concentration was increased. The diameters of inhibition zone to Nano-TET were (1.5, 3.5, 9.5, 15.0 and 19.5) mm of the following concentrations (62.5, 125, 250, 500 and 1000)  $\mu\text{g/ml}$ ; respectively.

In other hand, when we compare between Free-TET and Nano-TET to each concentration, the results refer to significance differences ( $P \leq 0.01$ ) in all concentrations, separately.

Table 5: **The inhibitory efficacy of Tetracycline against *E. coli***

| Concentration<br>( $\mu\text{g/ml}$ )      | Diameter of inhibition zone (mm) |                     | P value         |
|--------------------------------------------|----------------------------------|---------------------|-----------------|
|                                            | Tetracycline (Free)              | Tetracycline (Nano) |                 |
| <b>0 (Control)</b>                         | $0 \pm 0.00$                     | $0 \pm 0.00$        | <b>1.000</b>    |
| <b>62.5 (<math>\mu\text{g/ml}</math>)</b>  | $0.5 \pm 0.02$                   | $1.5 \pm 0.06$      | <b>0.0001 *</b> |
| <b>125 (<math>\mu\text{g /ml}</math>)</b>  | $1.5 \pm 0.42$                   | $3.5 \pm 0.23$      | <b>10.0019*</b> |
| <b>250 (<math>\mu\text{g /ml}</math>)</b>  | $5.5 \pm 0.06$                   | $9.5 \pm 0.97$      | <b>0.0020*</b>  |
| <b>500 (<math>\mu\text{g /ml}</math>)</b>  | $10.5 \pm 0.08$                  | $15.0 \pm 0.21$     | <b>0.0001 *</b> |
| <b>1000 (<math>\mu\text{g /ml}</math>)</b> | $12.5 \pm 1.04$                  | $19.5 \pm 0.89$     | <b>0.0009 *</b> |
| <b>P value</b>                             | <b>0.0000 *</b>                  | <b>0.0000 *</b>     |                 |
| <b>LSD</b>                                 | <b>3.571</b>                     | <b>1.804</b>        |                 |

The numbers refer to mean  $\pm$  Standard Deviation

\* refers to high significance differences (  $P \leq 0.01$  )

Through the results we can conclude that improvement of tetracycline effect by carry it with ZnO. The reason for the close difference between free-tetracycline and nano-tetracycline is that the loading rate of nano-tetracycline is 95.91% compared to free-tetracycline 100%.

Tetracycline is a broad-spectrum antibiotic that has been widely used to combat bacterial infections since its discovery in the 1940s. However, the emergence of bacterial resistance has significantly reduced its clinical efficacy [15]. Recent advancements in nanotechnology have introduced hybrid tetracycline nanoparticles designed to overcome resistance mechanisms and enhance antibacterial activity [16]. This discussion focuses on the antibacterial effectiveness of free tetracycline (TC) and nanohybrid tetracycline (Nano-TC) against *Staphylococcus aureus* and *Escherichia coli*, two clinically significant bacterial pathogens.

Tetracycline functions by inhibiting bacterial protein synthesis through its reversible binding to the 30S ribosomal subunit, thereby preventing the attachment of aminoacyl-tRNA to the ribosome [17]. This action results in the inhibition of bacterial growth, making tetracycline a bacteriostatic rather than a bactericidal agent. While it has been effective against both Gram-positive and Gram-negative bacteria, resistance mechanisms have increasingly limited its effectiveness [18].

The resistance of *S. aureus* and *E. coli* to tetracycline has been attributed to several mechanisms, including efflux pumps, ribosomal protection proteins, and enzymatic inactivation [19]. Efflux pumps, such as those encoded by the tet(A–E) genes, actively transport tetracycline out of the bacterial cell, reducing its intracellular concentration [20]. Ribosomal protection proteins, encoded by tet(M) and tet(O) genes, alter ribosomal conformation, preventing tetracycline binding [21]. Additionally, some bacteria produce enzymes that degrade tetracycline, further decreasing its efficacy [22].

Several studies have investigated the antibacterial effects of free tetracycline (TC) and nanohybrid tetracycline (Nano-TC) against *S. aureus* and *E. coli*. Free TC exhibits moderate inhibition against *S. aureus* and *E. coli*, with minimum inhibitory concentrations (MICs) typically ranging from 0.5 to 4 µg/mL for susceptible strains [23]. However, resistant strains often require significantly higher concentrations or show complete insensitivity to treatment [24].

Nanohybrid tetracycline (Nano-TC) has been developed to enhance drug delivery and combat bacterial resistance. Studies indicate that Nano-TC demonstrates a significantly lower MIC compared to free TC due to improved cellular uptake and prolonged retention within bacterial cells [25]. This enhanced efficacy is primarily attributed to the nano-formulation's ability to bypass efflux pumps and deliver the drug directly to intracellular targets [26].

In an experimental comparison, Nano-TC exhibited MIC values of 0.25–1 µg/mL for *S. aureus* and 0.5–2 µg/mL for *E. coli*, whereas free TC required higher concentrations to achieve similar inhibitory effects [27]. Additionally, time-kill studies revealed that Nano-TC maintained bacterial

suppression for a longer duration compared to free TC, indicating a sustained antibacterial effect [28].

Several previous studies have demonstrated the superior efficacy of nanotechnology-based antibiotics. For instance, a study by Zhang *et al.* [29] reported that tetracycline-loaded silver nanoparticles exhibited an MIC reduction of up to 80% compared to free TC in resistant *E. coli* strains. Similarly, Singh *et al.* [30] found that Nano-TC formulations improved antibacterial effects against multidrug-resistant *S. aureus* isolates. These findings align with the current research, reinforcing the notion that nanotechnology-enhanced antibiotics are promising alternatives to traditional formulations.

However, some studies have reported variable results based on the type of nanomaterial used. For example, while silver-tetracycline nanoparticles demonstrated potent antibacterial effects, polymeric tetracycline nanoparticles exhibited a slower but sustained release profile, leading to extended bacterial suppression rather than immediate inhibition [31]. The variations in results suggest that further optimization of nanohybrid formulations is necessary to maximize their clinical potential.

## Conclusion

The findings discussed highlight the limitations of free tetracycline in treating bacterial infections due to the prevalence of resistance mechanisms in *S. aureus* and *E. coli*. Nanohybrid tetracycline formulations offer a promising approach to overcoming these limitations by enhancing drug delivery, and prolonging antibacterial effects. While preliminary research supports the efficacy of Nano-TC, further studies are required to evaluate its clinical applications, safety, and potential resistance development. The integration of nanotechnology into antibiotic therapy represents a crucial advancement in the fight against bacterial resistance and may pave the way for more effective treatment strategies.

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