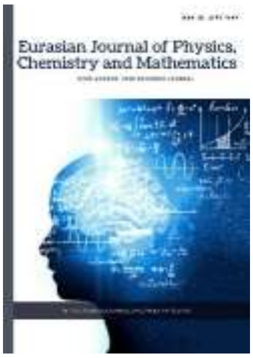


# Study of the effect of the compound nano-titanium dioxide prepared by Sol-Gel method through two stages on bacteria (E. coli and Streptococcus) as an antimicrobial agent.

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ABSTRACT

Using titanium tetrachloride ($TiCl_4$) as a starting material and altering the applied temperature in the range of ( 350 ,650nad ,850)°C, titanium dioxide ($TiO_2$) Nano-particles (NPs) are made using the sol-gel method. X-ray diffraction (XRD) and transmission electron microscopy have been used to evaluate the primary characteristics of the produced NPs (TEM). $TiO_2$ nanoparticles were primarily tested for their efficacy as an antibiotic and antibacterial agent using two different bacterial strains (E. coli and Streptococcus). According to XRD results, the titanium dioxide structure exhibits an anatase phase between 350 and 650 °C and a rutile phase at 850 °C. For anatase, the size of the particles is reported to be between (7.17 and 21.4 nm), while for rutile (30.41 nm). According to FTIR analysis, the majority of peaks are seen between 400 and 700 cm-1 as a result of bending and oscillation lengthening. The two phases of $TiO_2$ can use the antibacterial compounds against the two types of bacteria (E. coli and Streptococcus). The obtained results show that antibacterial agents made of $TiO_2$ in the anatase or rutile phases are effective.

**Keywords:** $TiO_2$ Phases, Sol-Gel Process, $TiCl_4$ Precursor, Antibacterial activity

## 1-Introuction

The study of materials and methods at the nanoscale via design, manufacture, characterization, and application constitutes the subject of applied science known as nanotechnology. It is a topic of research that is regarded as a subcategory for the technology in the scientific disciplines of colloidal knowledge, physics, chemistry, biology, and other domains, and it covers the investigation of phenomena and the doctrine of nanoscale materials[1, 2]. Titanium dioxide is one of the frequently utilized materials in a variety of applications because of its semiconducting, photocatalytic, energy-transforming, electrical, and gas sensing properties. Rutile, anatase, and brookite are the three different polymorphs of titanium dioxide crystals that exist in nature because to their redundancy[3, 4]. The capacity of NPs, which are simulated by UV radiation, to remove organic pollutants from various media is heavily researched. Examples include titanium dioxide (TiO2), a semiconductor with a wide energy band gap. TiO2 can also be used as a coating[5]. The sol-gel method, which will be used in this study, has a number of benefits, including ease of use and simplicity of the equipment, low energy costs, high purity and greater homogeneity of the material, and the ability to realize multi-component deposits in a single operation[6]. Due to its crystal structure and greater band gap of 3.2 eV compared to rutile's 3 eV, the anatase phase is particularly suitable for antibacterial applications. These NPs offer the advantages of being easily accessible, affordable, and low-toxic. The ability of semiconducting TiO2 to produce electron hole pairs when exposed to light with an energy larger than its band gap is crucial for the removal of numerous organic environmental contaminants. At the interface between the semiconductor and the solution, they may be utilized in several reduction processes[7] The two-phase titanium dioxide nanoparticles produced by a modified sol-gel technique are discussed in this work in relation to the effect of calcination temperature on the phase shift of these particles. The homogeneity of the gel is a function of the chemicals' solubility in the solvent. XRD and TEM instruments are used to characterize particles. In order to create $TiO_2$ NPs with two phases of anatase and rutile and test them against a range of diseases, including E. coli and Streptococcus bacteria, the sol-gel process is used in this article. $TiO_2$ NPs were examined for antibacterial activity on gram-positive and gram-negative bacterial cultures.

## 2- Experimental section

### 1-2 Materials and method for preparing and imposing nanoparticles

The Sol-Gel technique was utilized to create $TiO_2$ nanoparticles, and the processes are as follows: 1 cc of titanium tetrachloride (99.99 percent $TiCl_4$) was added using a graduated burette. The two solutions were combined with steady stirring but without heat after the production of white fumes from the hydrolysis of titanium tetrachloride was observed during the addition procedure (15 ml) of pure ethanol at room temperature (25) was noted. Use a magnetic mixer to blend for an hour and a half at room temperature to create a homogenous yellow light solution. After stirring continuously until the solution's pH (acidic) reaches (pH = 1.4), the homogeneous mixture is baked for 1.5 hours at a low temperature.

## 3- Results and Discussion

### 1-3- X-ray diffraction (XRD) test

(XRD) patterns of calcined titanium oxide ($TiO_2$) at three different temperatures (350, 650, and 850 °C) .The structure and the phases of $TiO_2$ NPs have been determined by X-Ray Diffraction analysis. Fig.1 shows that almost all of the crystal type in spectrum is anatase at (350ºC, 650 ºC). The intensity peaks of titanium oxide $TiO_2$ polycrystalline in anatase phase oriented at diffraction angles 2θ at (25.58˚, 37.16˚, 38.12˚, 38.87˚, 48. 31 ˚, 54.21 ˚, 55.38 ˚, 62.33 ˚, 69.11 ˚ , 70.65 ˚, 75.32 ˚ and 76.38 ˚ with diffraction planes (011), (013), (004), (112), (020), (015), (121), (123), (116) , (220) , (125), and (031). which is in agreement with the standard card (JCPDS 15-4609).The same figure illustrates the peaks of titanium oxide TiO2 polycrystalline in rutile phase at(850ºC)oriented at diffraction angle 2θ (27 .65 ˚, 36 .33 ˚, 39 .44 ˚,41 .48 ˚, 44 .37˚, 54 .66 ˚, 57 .008 ˚ , 63.03 ˚, 64 .42 ˚ ,69 .36 ˚,70 .11 ˚, 72 .79 ˚ and 76 .81 ˚) with diffraction planes (110), (011), (020), (111), (120), (121), (220), (002), (130), (013), (112), (131) and (022) respectively, which is in agreement with the standard card (JCPDS 08-5492).In order to create TiO2 nanoparticles, the following procedures were performed. The results of the X-ray analysis are consistent with those of the American Society of Testing and Materials (ASTM) and are in strong agreement with those shown in[1, 8][1,2]. According to the previous findings, as temperature rises, particle agglomeration causes the X-ray diffraction intensity to increase and the crystalline phase of $TiO_2$ to change from anatase to rutile. Additionally, it can be shown that the $TiO_2$ calcite at 850℃ diffraction peak narrows with increasing crystallite size. Based on Scheler's equation, the mean particle sizes for the anatase and rutile phases in this study were calculated to be between 7.17 and 21.4 nm and 30.41 nm, respectively[8].

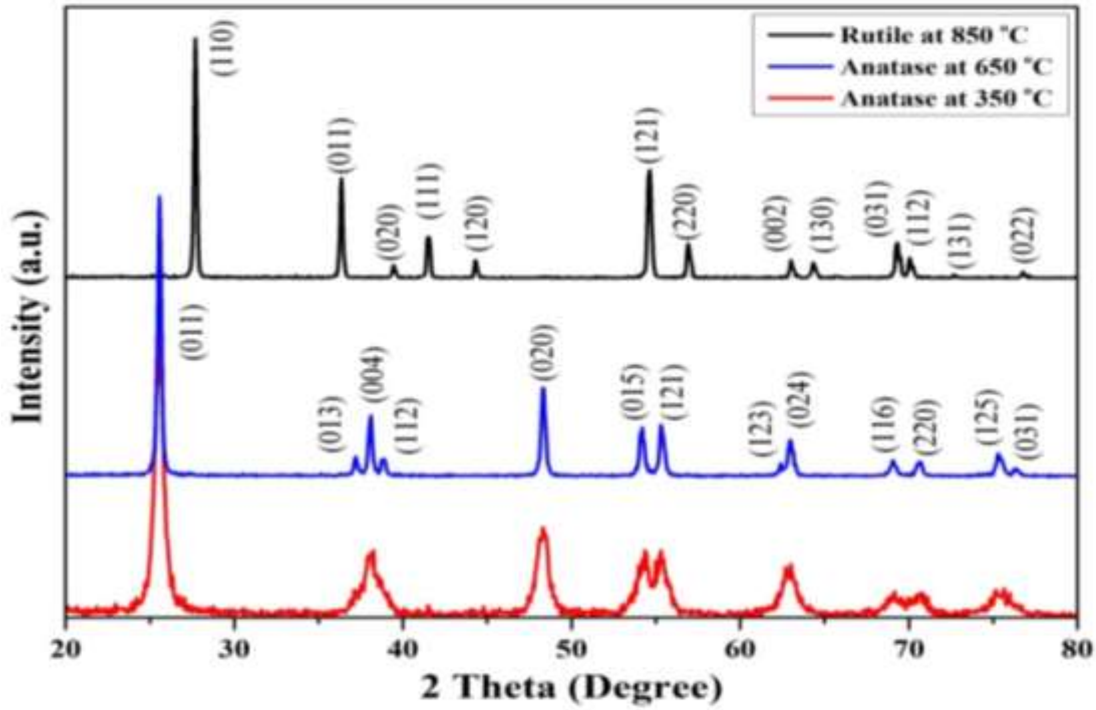



**Figure (1) the X-ray diffraction of calcined titanium oxide ($TiO_2$) at three different temperatures (350, 650, 850 °C).**

2-3 **Elemental analysis: FTIR**

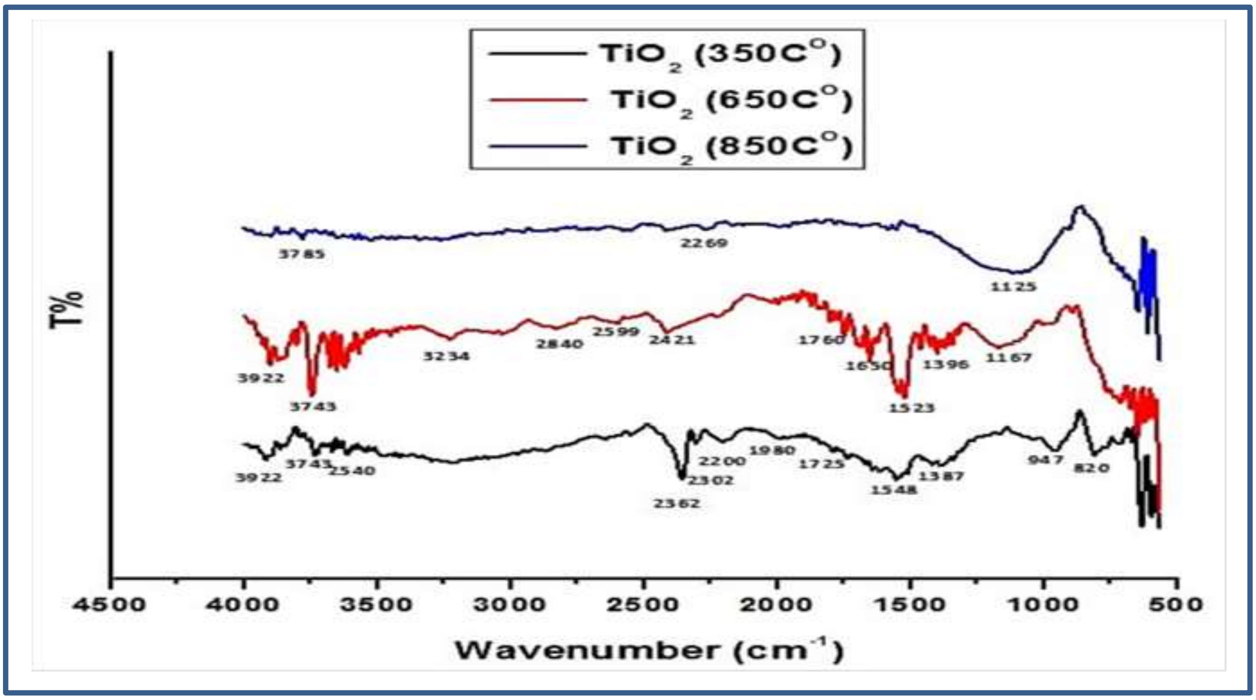



**Figure (2): FTIR spectra of all synthesized nanoparticles at different temperatures.**

After heating $TiO_2$ to 350 degrees Celsius, the bands linked to the stretching of the-CH2 and-CH3 groups were seen at roughly 2362 cm-1 in the spectra (black curve), those observed below 3000 cm-1 because of the ethyl groups' asymmetric and symmetric stretching vibrations, respectively [9]. The bands related to the C–O group represented by black and red curve at 1387 and 1167 cm-1 are present[10, 11]. while the asymmetric stretching of the OH groups is shown by a strong intensity band at 3743 cm-1. The strong bands related to the bending scissoring H-O-H vibration can be seen at 1548 cm-1 and 1523 cm-1 in black and red curves, respectively. The presence of weakly bound water determines the position and structure of this band, which is further supported by the band at 1650 cm-1 [12]. The bending vibrations of Ti–OH and Ti–O, O–Ti–O bonds are responsible for the signals between 1000 and 400 cm-1[13].

### 3-3) Results of Transmission Electron (Microscopy (TEM)

Results from transmission electron microscopy (TEM) show the dimensions and shapes of the anatase and rutile phases of TiO2 nanoparticles that were made using TiCl4 as a starting material and the Sol-Gel method. The size and shape of TiO2 NPs in the Anatase phase were measured using a TEM using hexagonal uniform and spherical homogenous particles, and the results were consistent with the reference[14]. Such particles can affect the local paint thanks to the homogeneities of TiO2 NPs. The photo-catalytic properties of the anatase phase, which are exploited in self-cleaning surfaces and antibacterial agents, will be utilised in paint. The TEM pictures of the anatase phase and TiO2 Rutile of TiO2 NPs at various sizes in the range of (200) nm are shown in Figures a-3 and a-4. Due to their properties, they can be used in paint applications. accumulation becomes significant, . Because the initial particle accumulation of aggregating is within the same size of crystalline, TiO2 NPs have shown non-uniformly of the particle shape. The particles may expressively accumulate at calcination temperatures and result in minimal development. At hight temperatures at 350, 650, and 850 °C , It was discovered that increasing the calcination temperature causes the size of the particles and the crystal size of the TiO2 particles to rise. This is due to an increase in crystal accumulation, which resulted in a decrease in surface area and an increase in crystal size respectively, and these results are in agreement with established norms[15]. Such results were predicted by the X-Ray diffraction studies, which showed that the anatase phase's particle size was smaller than the rutile phase's. it shows the statistical distribution of the particle diameters ($TiO_2$ Anatase) and it appears from the drawing that the nanoparticles' diameters are centered at (42.43) nm, as seen in picture (b-3). it shows the statistical distribution of the particle diameters ($TiO_2$ Rutile) and it appears from the drawing that the nanoparticles' diameters are centered at (50.08) nm, as shown in Figure (b-4)

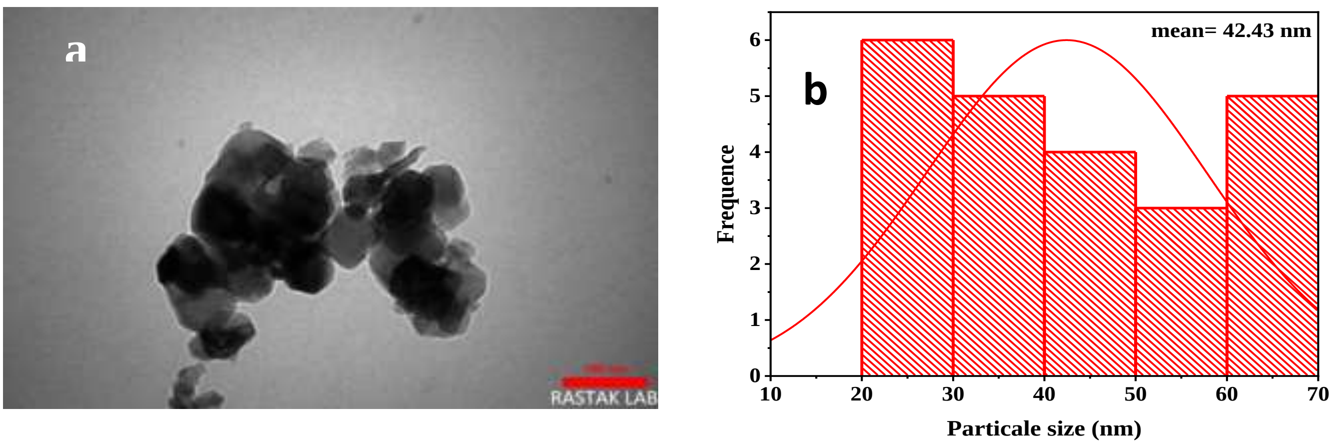
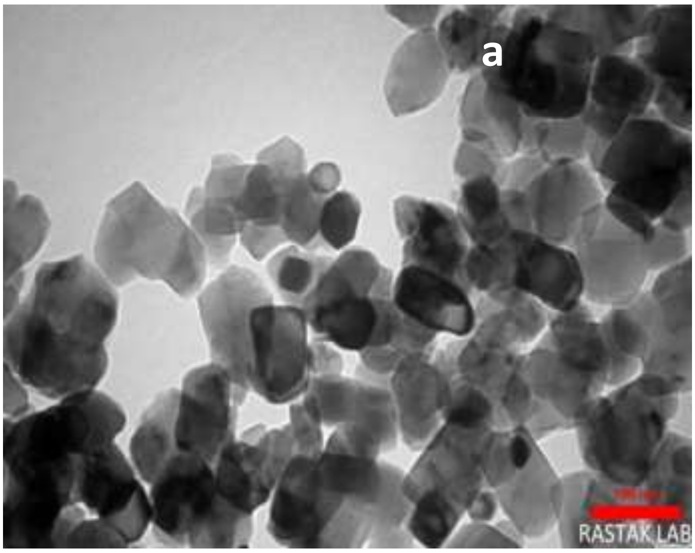



Figure 3. (a) Transmission electron microscopy (TEM) image of ($TiO_2$ Anatase) at calcined temperature (650 °C). (b) Statistical distribution of particulate matter ($TiO_2$ Anatase) at a wide



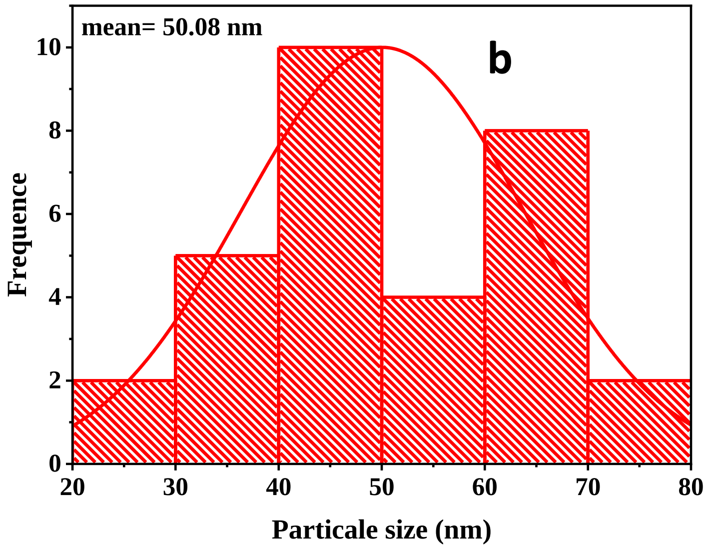




Figure 4. (a) Transmission electron microscope (TEM) image of (TiO2 Rutile) nanoparticles at calcined temperature. (850°C), (b)Statistical distribution of particles (TiO2 Rutile) at scale.

**4-Antimicrobial activity determination**

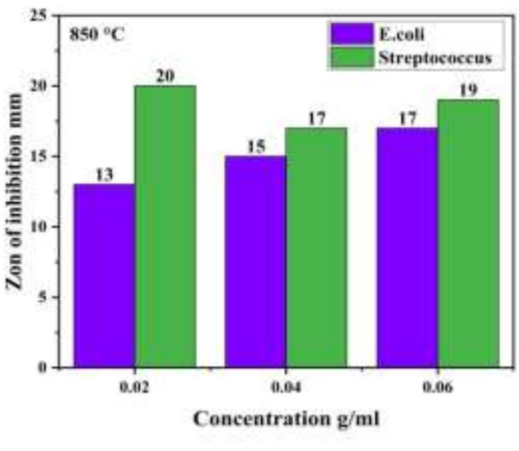
The fine diffusion method was used to investigate the antibacterial activity of $TiO_2$ NPs using a piercing gel, 6 mm and 9 mm diameter circles were created on a nutrient agar plate. Each single stress swab is inoculated onto a designated plate using a sterile cotton swab. Using different models of micropipettes, $TiO_2$, (20, 40 and 60) µg/mL and (40, 60) µg/mL nanoparticles precision transferred every minute on the entire plate in addition, using microcentre plates of continuous control water. Subsequently, polished at a custom 37°C for 24 h, the area of differentiated phases of growth was inhibited.

**4- 1- Antimicrobial test**

A Gram - positive of bacteria (Streptococcus), besides Gram-negative bacteria E. coli have been selected as a classical bacterial pollutant of work. strains of Bacteria accomplished thru Assessment in addition to the Laboratory of Diagnostic in Hospital of Baquba Teaching, Diyala/ Iraq. Bacteria were sub-cultured on nutrients agars ( N. agars) using the will-diffusion method technique, in addition to preparing two types of plates of preoccupations in dissimilar (0. 02, 0.04, 0.06) g/ml with 100 µl, and hatched at 37oC for 24h. Besides afterward display capacities inhibiting surrounded and measured by mm.

**4-2 Result Activity of Antibacterial Study**

According to several studies, According to various studies, metal oxides have a positive charge while microorganisms have a negative charge; this generates electromagnetic attraction between microorganisms and metal oxides, resulting in oxidation and, eventually, death of the microorganisms.; which leads to oxidization and finally death of microorganisms. They cause pits or holes of bacterial cell walls that could be associated with internalized particles, leading to increased permeability and cell death. $TiO_2$ nanoparticles interact with bacterial cells at a higher level than bigger particles due to their small size and high surface-to-volume ratio, which results in high antimicrobial properties. An experiment was conducted to test the antibacterial activity of $TiO_2$ nanoparticles produced at three different current concentrations. Antibacterial activity has been demonstrated to be decent. An agar fine distribution approach was used to investigate the antibacterial activity of $TiO_2$ nanoparticles (synthesis on three different amounts of current) against E. coli and Stretococus for three different concentrations of $TiO_2$ nanoparticles (0. 02, 0. 04, and 0. 06) g/ml. They are distinguished by the antibacterial well diameter condition, whereas the model is in the form of (6 mm and 9 mm), which incorporates antibacterial well models, but if the anti-bacterial diameter is equal to or minus 6mm and 9mm, the sample contains minor antimicrobial properties. Complete models exhibited increased anti-bacterial activities for circular diameters greater antibacterial abundance than 6mm 9 mm , as a result shown in Table 4, The $TiO_2$ nano-particle demonstrated actual activity against a gram-positive test strain. This difference in resistance to nano-particles between gram-negative and gram-positive bacteria can be explained by the fact that sold intermediates may prefer closure interfaces of postponed nano-particles and gram-positive microbial cells, which may be better associated with the microbial cell outside, originating from a building that is altered then crushed, resulting in cell death[16]. The $TiO_2$ nanoparticles were found to be very active on the Gram-positive strains tested. This difference in sensitivity between gram-negative and gram-positive bacteria to nanoparticles could be explained by the fact that the liquid medium likely favors close interaction between suspended nanoparticles and gram-positive microbial cells, which could better attach and anchor to the microbial cell surface, resulting in cell death due to structural alterations and damage. Gram-positive bacteria have a thick wall made up of many layers of peptidoglycan polymer and only one membrane (plasma membrane). Gram-negative bacteria have a cell wall with two cell membranes: an outer membrane and a plasma membrane, but only a thin layer of peptidoglycan. The addition of the gram-negative bacteria cells' outer membrane affects the permeability of numerous chemicals. If specific requirements are met. In addition, gram-negative bacteria are more resistant to a variety of chemical agents than gram-positive bacteria. Furthermore, due to the limited amount of peptidoglycan in gram-negative bacteria's cell walls, they are more prone to mechanical fracture[17]. It indicates that as the external till capacity proportion increases, so does the antibacterial activity of nanoparticles, which decreases as the nanoparticle size decreases[18].



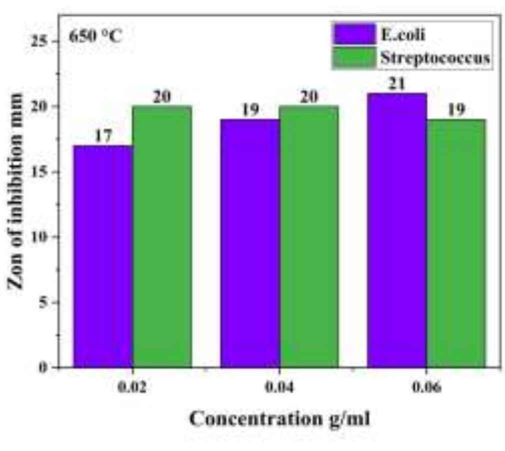
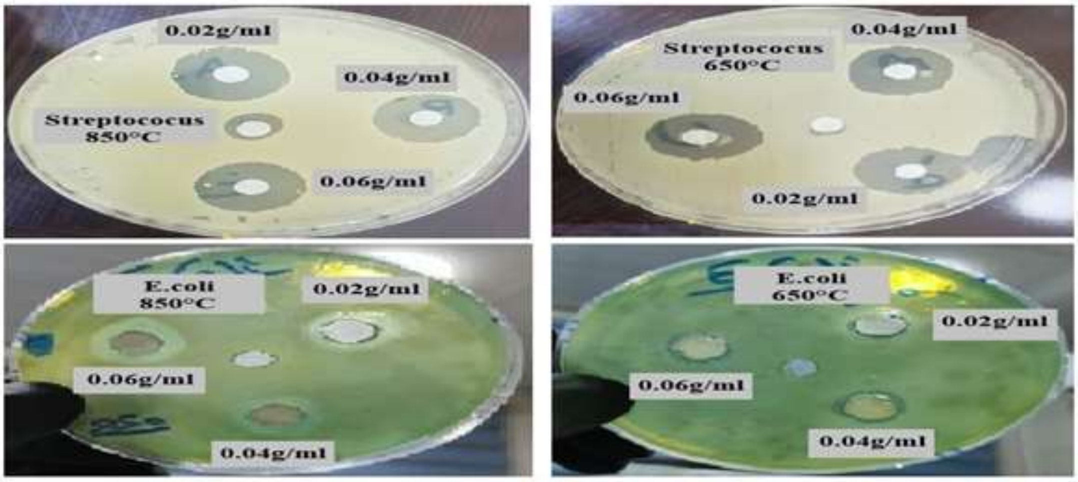



**Figure (5); Antibacterial activity of $TiO_2$ 650℃ Anatase and $TiO_2$ 850℃ Rutle phases) with different diameters of the wells, red (6mm) and blue (9mm) at (2, 4, and 6) mg/ml using Well-Diffusio.**




**Figure(6): Photograph of antibacterial activity of $TiO_2$ against E. coli and Streptococcus.**

**Table (1): Well-Diffusion antibacterial activity of $TiO_2$ (Anatase and Rutile)**

| Temperature °C | Phase TiO2 | Concentratio g/ml | Inhibition 9mm | Zone of growth 6mm |
|---|---|---|---|---|
| | | | E. coli | Streptococcus |
| 650 | Anatase | 0.02 | 17 | 20 |
| | | 0.04 | 19 | 20 |
| | | 0.06 | 21 | 19 |
| 850 | Rutile | 0.02 | 13 | 20 |
| | | 0.04 | 15 | 17 |
| | | 0.06 | 17 | 19 |

## 5- Conclusion

We succeeded in achieving different phases of $TiO_2$ nanoparticles by only controlling the calcination temperature during the sol-gel synthesis process, X-ray diffraction (XRD) confirmed the two-phase formation of $TiO_2$ nanoparticles and that the effect of calcination temperature on the crystal body and the particle size . will be handled on the scale of the particle size for the rutile phase of $TiO_2$ NPs in the range of (40-60) nm liquefied in 'ethanol' using as a self-cleaning tests and the large-size for the anatase phase of $TiO_2$ NPs in the range of (15-20) nm dissolved in water appropriate for the testing of medicinal drug agents.. Due to the accumulation of crystallites, which is crucial to the formation of big particles, the usual size of $TiO_2$ NPs particles increases with temperature. A "correlation" between the results of (XRD, TEM) representations occurs in the rutile phase. The fabric's crystal dimension should be taken into consideration to be 50 nm. FTIR of $TiO_2$ NPs in the range (400-1000) cm-1 revealed Ti-O and Ti-O-Ti bonds. Anatase nanoparticles appeared more efficiently than rutile nanoparticles when used as antisense against bacteria at different concentrations. The NPs of anatase arisen by extra efficacy of Nano-particles rutile using as a 'Gramothic antagonist' contrary to bacteria in addition through concentration of ,' 0.02, 0.02 and 0.06' g/ml.

**6-Acknowledgments**

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