Synthesis and Antibacterial Activity of Rutile-TiO_2 Nano Powder Prepared by Hydrothermal Process

Rashed T. Rasheed [1] **Sariya D. Al-Algawi** [2] **Zeena R. Rhoomi**[*]

(1) Applied Chemistry Division, School of Applied Sciences, University of Technology, and Baghdad, Iraq.

(2) Applied Physics Division, School of Applied Sciences, University of Technology, and Baghdad, Iraq.

zeena.rabah12@yahoo.com

Abstract

Rutile titanium dioxide (r-TiO_2) Nano powder has been synthesized by hydrothermal method in autoclave. The reaction took place between titanium tetrachloride ($TiCI_4$) and mixture solution consisted of deionized water and ethanol, in the ratio (3:7) respectively. The product has been dried and annealed at 400°C. The structure, morphology and the particle size of the Nano powder were investigated by X-ray Diffraction, Scanning Electron Microscopy (SEM), Atomic Force Microscope (AFM), FT-IR and UV/visible spectroscopy measurements. The effect of r-TiO_2 on gram-negative bacteria *Escherichia coli* (*E. coli*) and gram-positive bacteria *Staphylococcus* aurous (*S. aureus*) has been studied. This study showed that rutile TiO_2 Nano powder has efficient antibacterial activity, and can use as an antibacterial agent for different purposes.

Keywords: Titanium dioxide (TiO_2) nanopowder, hydrothermal method, antibacterial properties.

الخلاصة

حضر اوكسيد التيتانيوم النانوي نوع روتايل (r−TiO_2) بتقنية الحراره المائيه في الأوتكليف، حصل التفاعل بين رباعي كلوريد التيتانيوم ومزيج متكون من ماء لاايوني وايثانول بنسبة (7:3) على التوالي. جفف الناتج ولدن عند 400 °م. التركيب وطوبوغرافية السطح لاوكسيد التيتانيوم تم تشخيصها بواسطة قياسات حيود الاشعة السينية (XRD) ، المجهر الألكتروني الماسح (SEM), مجهر القوة الذرية (AFM) ومطيافية الأشعة تحت الحمراء (FT−IR) والأشعة فوق البنفسجية/المرئية (UV/Visible). تم دراسة تاثير الاوكسيد المخصر على نوعين من البكتريا الغرامية السالبة (*Escherichia coli*) والموجبة (*Staphylococcus* aurous). بينت الدراسة ان اوكسيد التيتانيوم النانوي نوع الروتايل (r−TiO_2) له فعالية مضادة للبكتريا ويمكن استخدامه كمضاد بكتيري لمختلف الاغراض.

الكلمات المفتاحية: اوكسيد التيتانيوم، الحراره المائية، خصائص المضاد البكتيري.

1. Introduction

Titanium dioxide (TiO_2), also known as titanium belongs to the family of transition metal oxides. It is the naturally occurring oxide of titanium. It is n-type semiconductor material with wide band gap (Eg= 3 - 3.3eV) Iatsunskyi 2015, TiO_2 has good stability, high transparent in a visible region and absorption in the ultraviolet region and low conductivity, have high refractive index, low cost, good chemical stability, nontoxicity, mechanical hardness, novel optoelectronic properties and easy availability Nolan (2010) These advantages make TiO_2 a material in solar cells, fuel cell, chemical sensors for hydrogen gas evolution, a pigment, self-cleaning surfaces, environmental purification applicationsSharmila (2014) resistance to photochemical, chemical erosion and having a lot of interesting properties from fundamental Sarah Skhtar(2016) .Titanium dioxide occurs in nature in three various polymorphs: brookite, rutile and anatase. Rutile is a mineral composed primarily of titanium dioxide it is reddish brown but sometimes yellowish. Rutile and anatase are both tetragonal in structure whereas, brookite exists in a rhombohedral structure Bakri (2017), Nolan, (2010), **,** Rutile is the most stable phase of titanium dioxide, while anatase and brookite are metastable phases. However, anatase is the general favor for solution phase preparation Luis(2011). Anatase and brookite are a metastable phase and readily transform to rutile when heated MAJEED A. (2012) Several methods have been reported in the literature to prepare TiO_2, including the hydrolysis of acidic solutions of Ti (IV) salts, oxidations of $TiCl_4$ on gaseous phase D. Reyes(2008), hydrolysis of titanium alkoxides, sputtering, chemical vapor deposition and sol-gel process Paola(2013). Among these techniques, the sol-gel technique has emerged as one of the most promising technique as this method produces samples with a good homogeneity at low cost Shuxi Dai (2010).They were usually found that different routes often produced different results. Even for the same route, using a different amount of the starting materials, the obtained powder size is different Muaz (2015). Finally, the antibacterial effect of those nanoparticle suspensions was investigated, both qualitatively and quantitatively, using *Escherichia coli*, as gram- negative bacterium and *Staphylococcus aurous*, as gram- positive bacterium.

2. Theoretical

In the present work, we have prepared TiO_2 nano powder using $TiCl_4$ as a precursor. The grain size for that peak alone calculated, using the Debye- Scherer formula Sarah Akhtar (2016):

$$D = k\lambda / \beta\cos\theta \quad \text{………} \quad (1)$$

Where k is the constant (0.9), λ is the wave length of X-ray (1.54 nm), β is the full width half maximum (FWHM) of the peak and θ is the reflection angle.

And the optical absorbance coefficient α of a semiconductor close to the band edge can be expressed by the following equation:

$$\alpha = A\ (h\nu\text{-}Eg)\ n/h\nu \quad \text{…………} \quad (2)$$

Where α and Eg are the absorption of coefficient and band gap respectively, A is constant, n depends on the nature of the transitions, h is Plank constant and v is vibration of light Mua (2015).

3. Materials used

All reagents used were of analytical grade purity and no further purification was done before use. Titanium tetrachloride ($TiCl_4$), purity 99.9%; ethanol (EtOH) grade, purity 97%.

Synthesis of TiO_2 Nano powder

TiO_2 nano powder was prepared via hydrothermal method using titanium chloride ($TiCl_4$), ethanol (EtOH) and deionized water as the starting materials. Five milliliters of $TiCl_4$ were added slowly under stirring into a round bottom flask putting in an ice bath. The round bottom flask has a mixture solution consisted of deionized water and ethanol, in the ratio (3:7) respectively. The process was done under the fume hood. After 30 minutes with vigorous stirring on the magnetic stirrer, the colorless solution poured into a 50 ml Teflon-lined stainless-steel autoclave. The autoclave was sealed and placed in an oven at 200°C for 6 h. Then, the autoclave was allowed to cool to room temperature naturally. The white precipitate ($Ti(OH)_4$) was washed with distilled water (about 3 times), and collected by centrifugation, washed with ethanol (2 times) and annealed at (400°C). The reaction took place according to following steps:

1. $TiCl_4 + 3CH_3CH_2OH + H_2O \xrightarrow{\text{ice bath/stirring}} Ti(OCH_2CH_3)_3(OH) + 4HCl$
2. $Ti(OCH_2CH_3)_3(OH) \xrightarrow{\text{autoclave}} Ti(OH)_4 + 3CH_2CH_2$
3. $Ti(OH)_4 \xrightarrow{\text{annealing}} TiO_2 + 2H_2O$

The final equation is:

$$TiCl_4 + 3CH_3CH_2OH + H_2O \xrightarrow[\text{2.autoclave 3. Annealing}]{\text{1. Ice bath/stirring}} TiO_2 + 4HCl + 3CH_2CH_2 + 2H_2O$$

Antibacterial activity of TiO_2 in the Dark.

Bacterial growth was performed in order to observe the effect of different TiO_2 nanopowder concentrations on strains of *E. coli* and *Staphylococcus aureus*. TiO_2 nanopowder was suspended at the concentration of 13.3 g/L. A hundred microliters of the suspension (125, 250, 750 and 1000 mg/ml) of r-TiO_2 suspended were then added to a sterile Petri dish. The Petri dishes were inoculated with *Escherichia coli* and *Staphylococcus aureus* and incubated at 37°C for 24 hours. After that, the diameter of the antibacterial circle of any r-TiO_2 suspended sample was measured.

3. Results and Discussion

The XRD is employed for the identification and understanding the crystalline growth nature of titanium dioxide structures prepared by the hydrothermal method. Calcination is a common treatment used to improve the crystallinity of TiO_2 powders F. Hanini (2013).

XRD patterns of TiO_2 powders annealed at 400 ºC are shown in figure 1. The Nano powder rutile structure was confirmed by sharp peaks obtained corresponding to the plane (110) at 2θ = 27.60 refer to the tetragonal structure belonged to rutile phase.

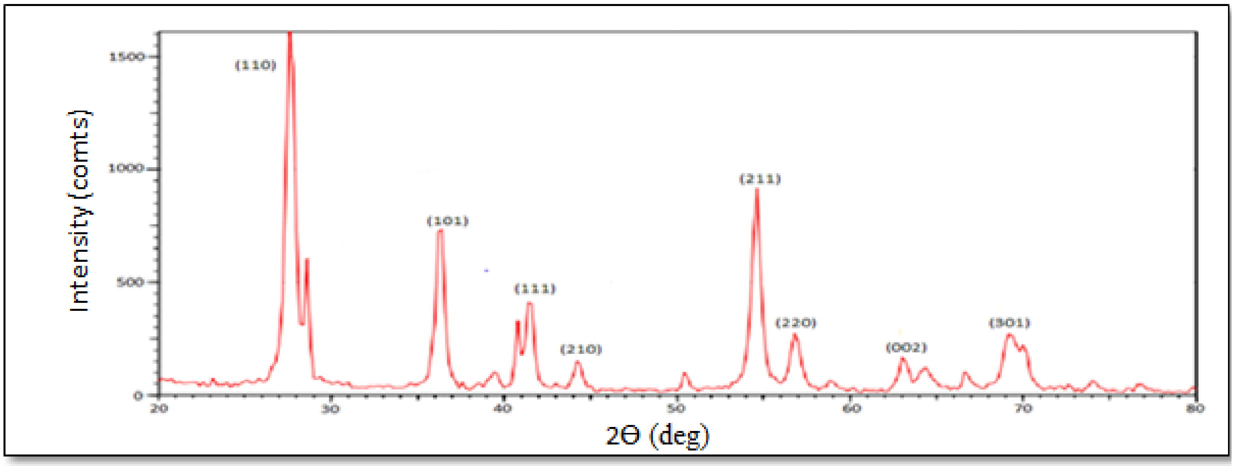


Figure (1). XRD patterns of TiO_2 annealing temperature at 400°C for 120 min.

The FT-IR spectrum (fig. 2) show that the peaks at 3380 cm^{-1} and 1620 cm^{-1} are characteristic of surface-adsorbed water and hydroxyl groups (stretching and bending vibration of the O–H group respectively) H. Esteban Benito (2014) These peaks are decreased and become smaller with increasing annealing temperature, corresponding to decreases the amount of water in samples with increasing annealing temperature and the Ti-O stretching become broader and more significant.

There is no peak at 2900 cm^{-1} for all spectra of titanium nano powder regarding C-H stretching band, which means all organic compounds were removed from the samples after wishing and annealing. The broad intense band saw below 1200 cm^{-1} and around 560-460 cm^{-1} is due to vibration, stretching and bending of Ti-O-Ti group respectively R. Sharmila Devi (2014), Rutile phase (r-TiO_2) of TiO_2 exhibit certain strong FT-IR absorption bands in the regions of 800−650 cm^{-1}T. A.M.Shehap (2016)

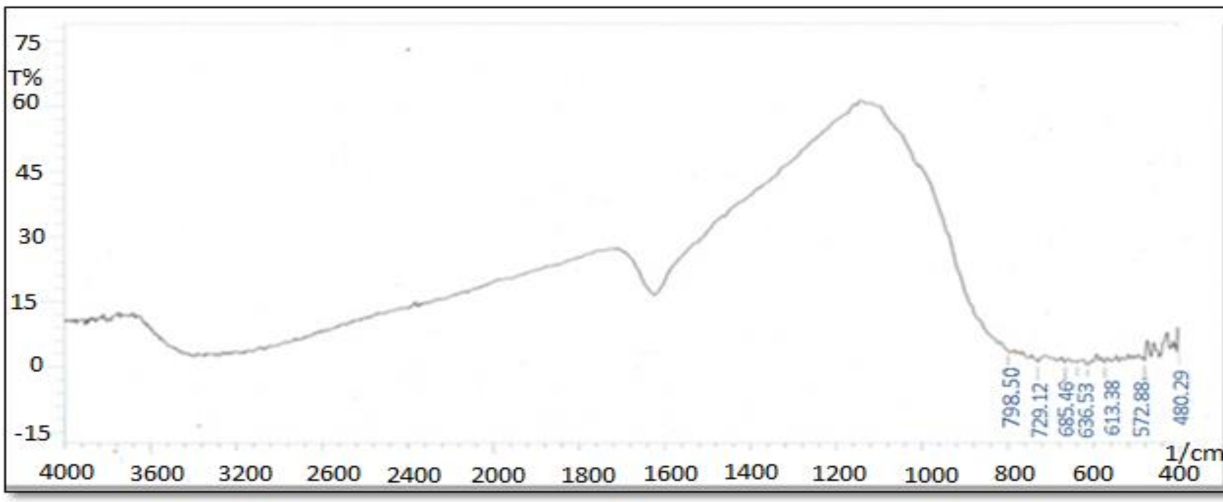


Figure (2). FTIR transmittance spectrum of TiO_2 annealing at 400°C for 120 min.

Figure (3) shows a typical two and three-dimensional AFM image and the granularity accumulation distribution chart of TiO_2 nano powder with annealing at 400°C. The average grain size found to be 74.5 nm.

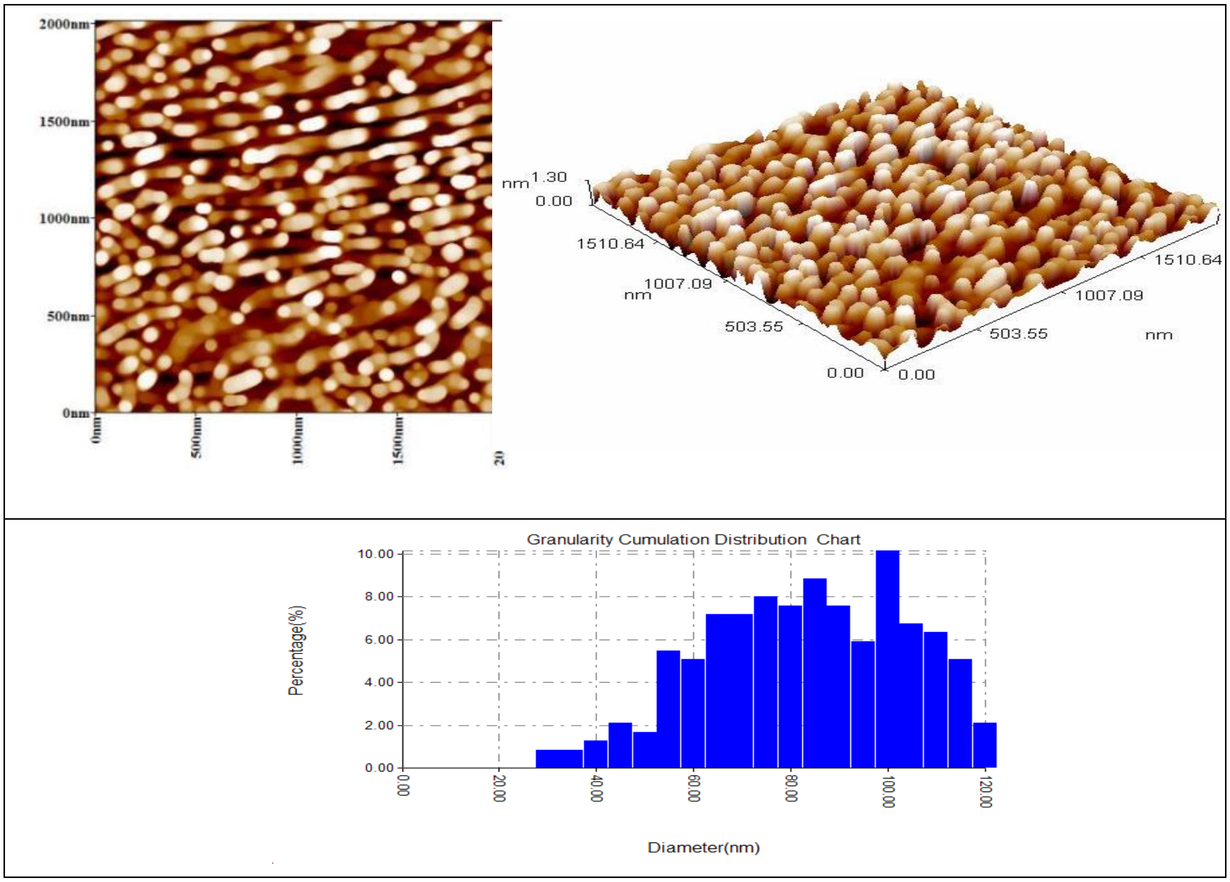


Figure (3). 2-D, 3-D dimensional AFM image and the granularity accumulation distribution chart of TiO_2 powders with annealing temperature at 400°C for 120 min.

Figure (4) shown the SEM images of TiO_2 that prepared by hydrothermal method at annealing temperature 400 °C, according to the morphology of TiO_2, there are nanosphere shapes. The SEM result revealed that the r-TiO_2 microspheres had a rough surface and were composed of many TiO_2 nano powder agglomerates.

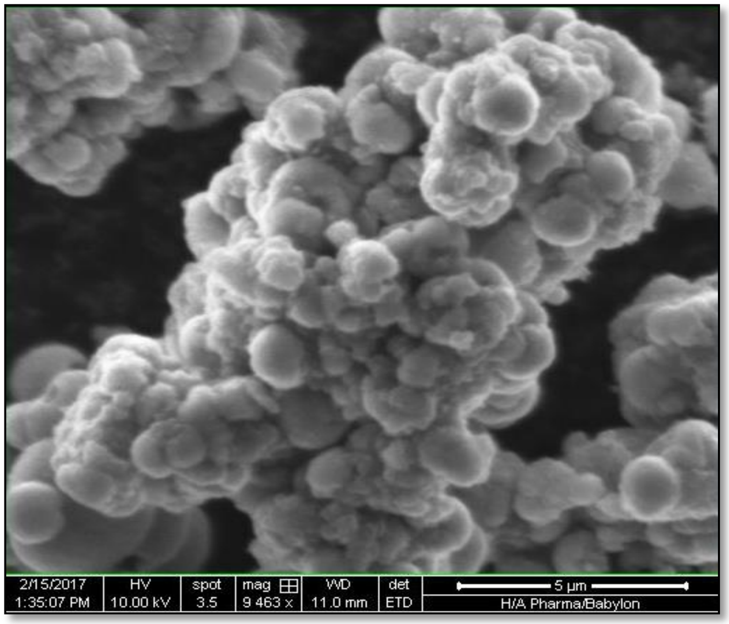


Figure (4). SEM image for TiO_2 nano powder at 400°C for 120 min.

figure (5) shows the UV-Visible of the optical transmittance curves as a function of the wavelength for the TiO_2 nano powder at annealing temperature 400°C for (120 min). Transmittance spectrum was measured in the wavelength range of 350 – 850 nm. As can be seen, an increase in the annealing temperature, which improves the optical transmission. Obviously, the nano powders are fully transparent in the visible region and a sharp fall in the UV region 300-400 nm. The TiO_2 nano powder spectra exhibit high visible transmittance, up to 85 % in the UV-Visible region for the TiO_2 nano powder, attributed to the quantum size effect Sharmila Devi (2014).

The optical band gap of the TiO_2 nano powder is found to be 3.2 eV. For the samples annealed at 400˚C which is larger than the value of (3.2 eV) for the bulk TiO_2. This can be explained because the band gap of the semiconductors has been found to be particle size dependent Muaz (2015). The band gap increases with decreasing particle size and the absorption edge are shifted to higher energy (blue shift) with decreasing particle size the absorption edge shifts towards lower energy side, indicating the decrease in the band gap. Indeed, a quantitative analysis of the absorption edge shifts (showed in the fig. 5) leads to a very good agreement with those of the band gap energies.

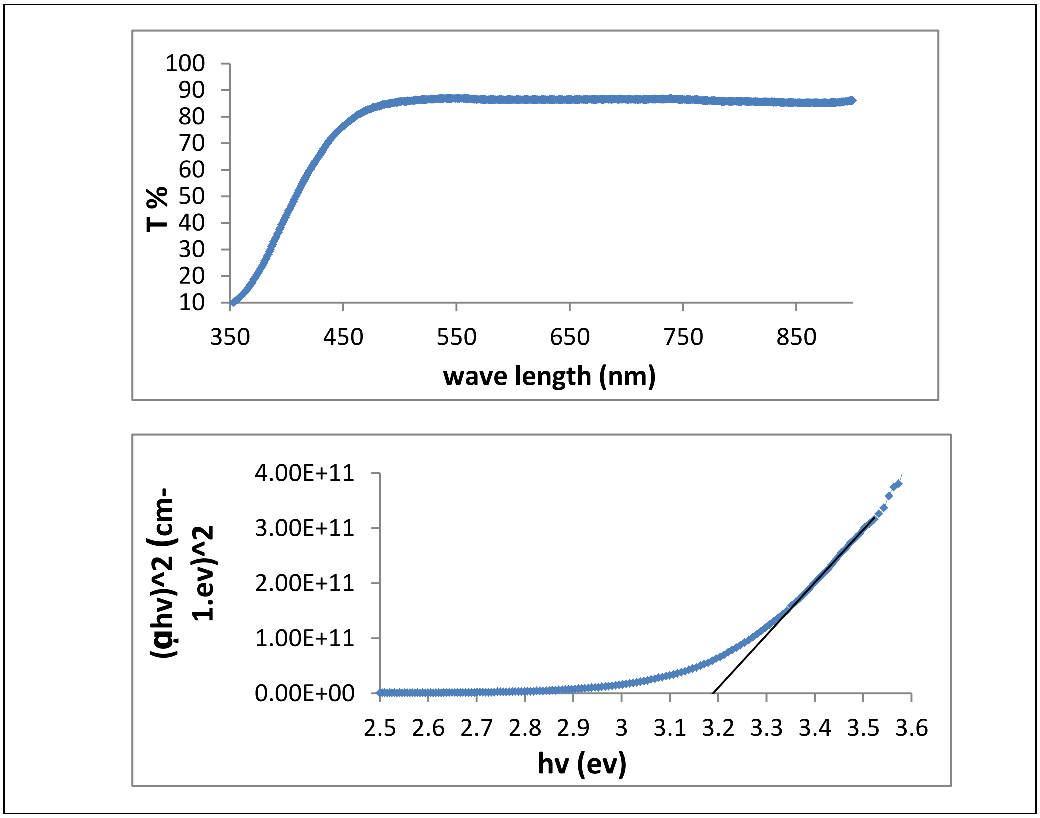
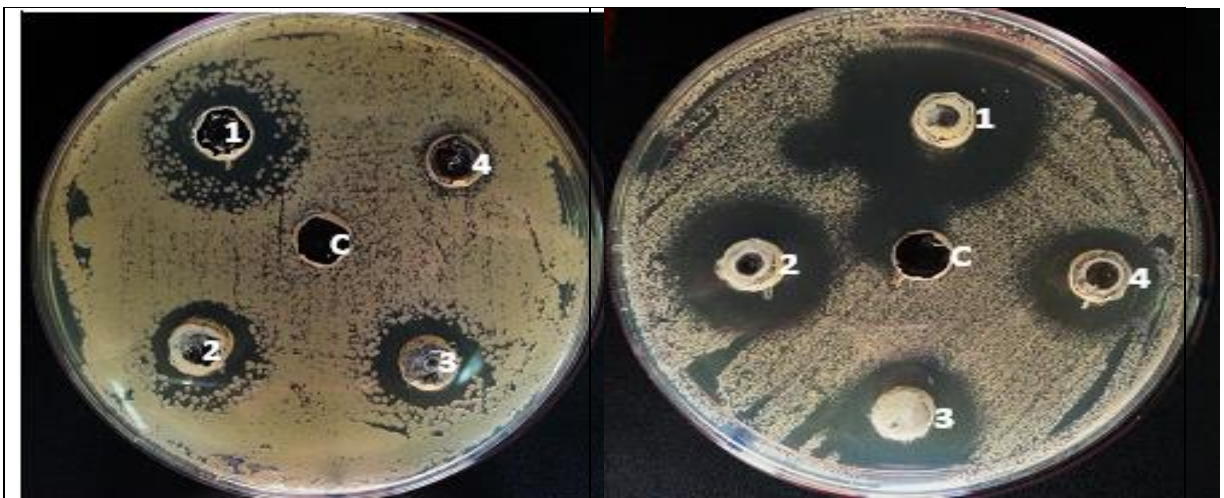


Figure (5). Optical Transmission as a function of wavelength for r-TiO_2 annealing at 400 °C for 120 min.

Antibacterial efficacy was determined on the basic of duplicates test results demonstrate that is generally agreed that the TiO_2 powder has stronger effect and more efficient on *Staphylococus aurous* with 10^3 mg/ml concentration than *Escherichia* coli at Annealing temperature (200, 400, 600, and 800°C). figures (6 and 7) are show the relationship between various concentrations of r-TiO_2 nano powder with *Staphylococcus aureus* and *Escherichia coli* bacteria living cells after 24h in 37°C of contact, The gram-positive bacteria have a relatively thick wall composed of many layers of peptidoglycan polymer, and only one membrane (plasma membrane). The gram-negative bacteria have only a thin layer of peptidoglycan and a more complex cell wall with two cell membranes, an outer membrane, and a plasma membrane Reddy (2002). It is well known that if the diameter of antibacterial circle of one sample is larger than 7 mm, it means that the sample has better antibacterial activity, however, if the diameter of antibacterial circle is equal to or less than 7 mm, it means that the sample has poorer antibacterial activity Zhao (2007). Table (1) against *S. aureus and E-Coli* shows TiO_2 powder at annealing temperature (200-800°C) in the form of zone-of- curb studies. Observed that the antibacterial activity increases with annealing temperature of the powder increase. This suggests that such annealing process may be able to turn any titanium powder to become ambientactive. The heat treatment improves the antibactarial activity of the TiO_2 powder Stimulate a preferential direction of TiO_2 rutile in (110) peak. the relationship between various concentrations of TiO_2 powders with *S. aureus and E-Coli* bacteria by agar well definition of as showing in figure (8) .It is observed that the inhibition zone was increase slightly as the concentricity of TiO_2 powder increase in all types of tested Pathogens, the 1000 µ g ml^{-1} were the perfect concentration of TiO_2 powder for inhibiting growth of both bacteria strains. This result showed that TiO_2 powder was effective for inhibiting both gram-positive and gram-negative bacteria Yage Xing(2012). The antibacterial activity against *S. aureus* was stronger than that against *E. coli* may be due to the difference structure and thickness of the membrane cell wall between *S. aureus* and *E. coli*.



E-coli	S. aureus

Figure (6). Photographs of anti-bacteria and different concentration of TiO_2 nano powder (1= 1000, 2= 500, 3= 250, 4= 125) µg/ml

Table (1): antibacterial screening of synthesized titanium dioxide nano powder

Organism	TiO_2 concentrations			
	1000 μg/ml	500 μg/ml	250 μg/ml	125 μg/ml
S. aureus	27.5	22	20	18
E. coli	24	20	16	12

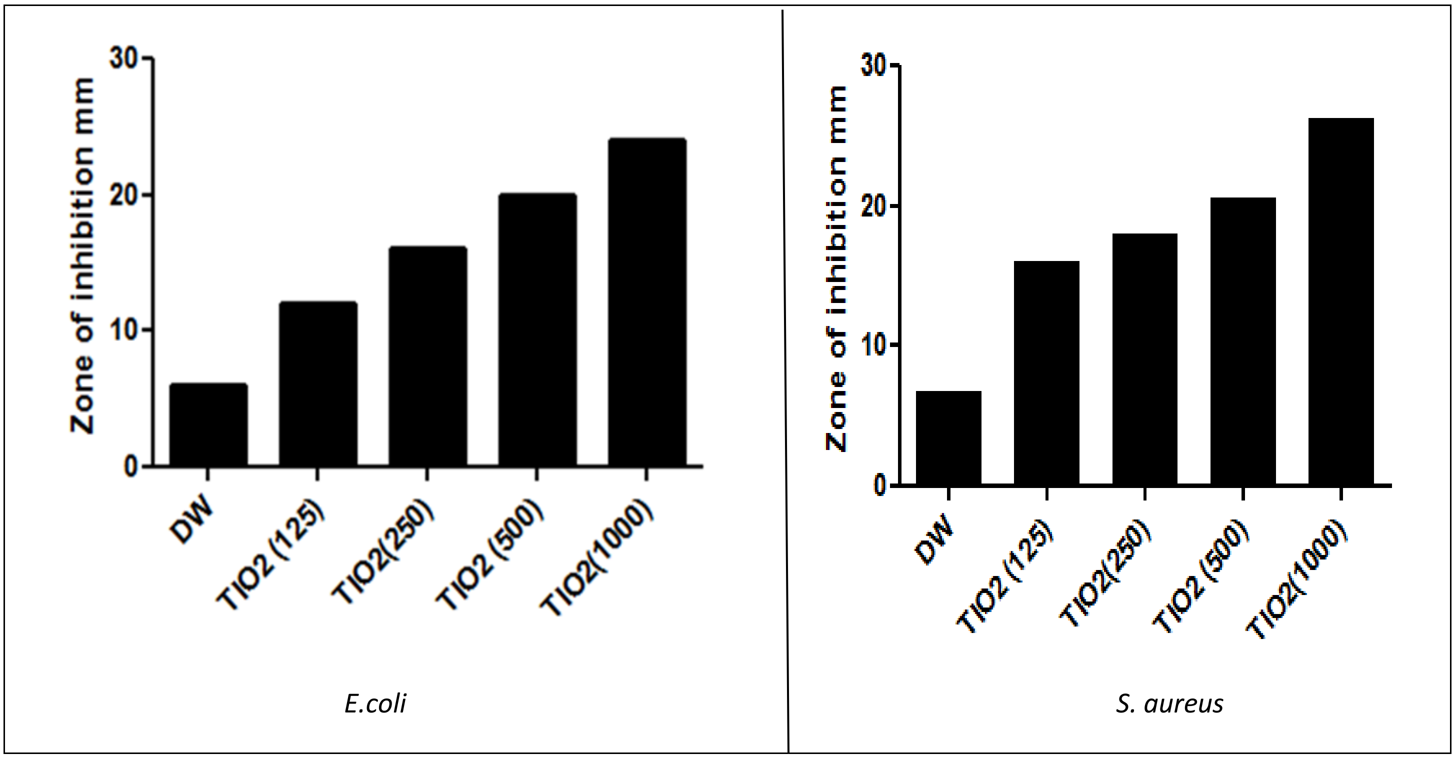


Figure (7). Antibacterial efficiency for the different TiO_2 nano powder concentration on anti-bacterial. *E.coli* , *S. aureus.*

Conclusions

The TiO_2 was a tetragonal rutile phase was confirmed by X-ray diffraction pattern with the sharp peak at $2\theta = 27.60$ [110] and 3.2eV energy band gap. Antibacterial activities were studied against gram negative and gram positive antibacterial (*Escherichia coli* and *Staphylococcus aurous*) respectively. The more efficient phase as antibacterial agent TiO_2 nano powder was effective and sensitive with *Staphylococcus aurous* more than *Escherichia coli*. The antibacterial efficiency increased when increasing the concentration of TiO_2 solutions.

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