

Synthesis of TiO₂ Nan particles by Using Sol-Gel Method and its Applications as Antibacterial Agents

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ABSTRACT

TiO₂ nanoparticles were prepared from TiCl₄ as a precursor with ethanol solution with 1:10 ratio in ambient atmosphere, without additive. Sol-gel synthesized has been dried and calcined at (550-600)°C. The structure, morphology and the particle size of the nanoparticles were investigated by X-ray Diffraction and Scanning Electron Microscopy (SEM). The optical properties were studied by UV-Visible Spectrophotometer. Results showed that the anatase phase was only in titanium dioxide powder up to 500. The average grain size of TiO₂ nanoparticles was obtained in the range of (3- 30) nm. The synthesized TiO₂ nanoparticles in 10⁻⁵ and 10⁻³ concentrations exhibited superior antibacterial activity with two types of bacteria, *Escherichia coli* (*E-coli*) and *Staphylococcus aureus* respectively. TiO₂ nanoparticles are more efficient as antibacterial agents with *Staphylococcus aureus* as compared with *E-coli*.

Keyword: Tio₂ Nanoparticles, Ticl₄, Sol-Gel Method, Tio₂ Phases, Antibacterial Properties.

تصنيع جسيمات ثنائي اوكسيد التيتانيوم النانوية باستخدام طريقة محلول
- جل وتطبيقاته كعوامل مضادة للبكتريا

الخلاصة

تم تحضير جسيمات ثنائي اوكسيد التيتانيوم النانوية من رباعي كلوريد التيتانيوم ومحلول الايثانول بنسبة 10:1 في الظروف الاعتيادية وبدون اضافات. المحلول- جل المصنع يجفف ويسخن الى (550-600) درجة مئوية, ومن ثم تم تشخيص التركيب ومورفولوجيا السطح والحجم الحبيبي للجسيمات النانوية باستخدام جهاز حيود الاشعة السينية والمجهر الالكتروني الماسح والخصائص البصرية (فجوة الطاقة) باستخدام جهاز مطيافية الاشعة فوق البنفسجية - المرئية. اظهرت النتائج طور (anatase) يتكون في مسحوق ثنائي اوكسيد التيتانيوم عند 500 درجة مئوية. معدل الحجم الحبيبي لجسيمات التيتانيوم النانوية التي تم الحصول عليها كانت بمدى (3-30) نانومتر. جسيمات ثنائي اوكسيد التيتانيوم المصنعة بالتراكيز 10⁻⁵ و 10⁻³ تتصرف بفعالية مضادة للبكتريا فائقة مع نوعين من البكتريا *Escherichia coli* (*E-coli*) و *Staphylococcus aureus* على التوالي. جسيمات ثنائي اوكسيد

التيتانيوم النانوية تكون اكثر كفاءة كعوامل مضادة للبكتريا مع *Staphylococcus aureus* كمقارنة مع *Escherichia coli* (E-coli).

INTRODUCTION

Titanium dioxide (TiO₂) Nanoparticles have been of interest in a wide range of applications such as photocatalyst [1], dye-sensitized solar cells [2], gas sensor [3], nanomedicine [4] and antibacterial agents [5]. TiO₂ exists in three polymorphic phases: rutile (tetragonal density, 4.12 g/cm³), anatase (tetragonal density, 3.894 g/cm³) and brookite [6], both anatase and rutile have tetragonal crystal structures but belong to different space groups [7]. TiO₂ nanoparticles have been synthesized using various methods such as hydrothermal [8], sonochemical [9], solvothermal [10], reverse micelles [11], and sol gel reaction [12] for those applications. Recently, sol gel process has been used for the preparation of TiO₂ nanopowder [13, 14]. Experimental results have shown that this method had successfully produced uniform size, unagglomerated state, high purity and homogeneous nanoparticles [15]. In comparison to other phases using anatase phase of TiO₂ nanoparticles with controlled diameters, the high homogeneity, higher activity, good morphology, large surface area and porosity is very suitable to be used as an antibacterial material and photocatalytic applications because of higher electron mobility, lower fixed dielectric and density [16-24]. In this study, the synthesis of anatase crystal type of TiO₂ nanoparticles by sol-gel method was reported. Furthermore, the aqueous solution containing TiO₂ nanoparticles was prepared through nanoparticle/ liquid mixing; this process is simple, active, and suitable for antibacterial application. The TiO₂ nanoparticles were characterized by X-Ray Diffraction (XRD) technique, UV-Vis. Absorption spectrophotometer, and Scanning Electron Microscope (SEM). Finally the antibacterial effect of those nanoparticle suspensions was investigated, both qualitatively and quantitatively, using *Escherichia coli*, a Gram- negative bacterium and *Staphylococcus aureus*, a Gram- positive bacterium.

EXPERIMENTAL PROCEDURE

Synthesis Method of TiO₂ Nan particles

14 ml Titanium Tetrachloride TiCl₄ (99.99%, BDH, England) was slowly added dropwise into 140 ml absolute ethanol CH₃CH₂OH (99.99%, GCC, U.K). The reaction was performed at room temperature while stirring under a chemical fume hood due to the large amount of Cl₂ and HCl evolved in this reaction. The resulting yellow solution was allowed to rest and cool back to room temperature as the gas evolution ceased. The pH of the solution was in the range of (1-2). The obtained suspensions were dried in an oven for several hours at 80 °C until amorphous and dried TiO₂ particles. The obtained powder samples were calcined for two hours in a box furnace at temperature ranging from (550 to 600) °C in an ambient atmosphere, the powder transform to TiO₂ nanoparticles in anatase phase.

Characterization of TiO₂ Nanoparticles

X-ray diffraction (XRD-7000 Shimadzu Maxima-a) 40 kV voltage and with current 20 mA was used to identify the crystalline phases and to estimate the crystallite size. The XRD patterns were recorded with 2θ in the range of 10 - 60° by step scanning, employing Cu tube with wavelength of Cu 1.54 Å. Scanning Electron

Microscope (SEM) model (TESCAN-VEGA/USA) with resolution 3nm at 30 kV and UV-Visible Spectrophotometer (Metertech SP 8001), in the range of (190-1100) nm, were used to study optical properties.

RESULTS AND DISCUSSION

The XRD pattern showed that this sample have five sharp peaks 2θ angle at 25.39°, 37.89°, 48.1°, 54.005° and 55.18° with (101), (004), (200), (105) and (211) diffraction planes, respectively, see Figure (1). This pattern also demonstrated anatase phase of TiO₂ nanoparticles with superior antibacterial effect activity. The mean size of anatase TiO₂ nanoparticles have been estimated from full width at half maximum (FWHM) and Debye- Sherrer formula as follows: [25]

$$D=0.89\lambda / B \cos\theta , \quad \dots (1)$$

Where, 0.89 is the shape factor, λ is the X-ray wavelength, B is the line broadening at half of the maximum intensity (FWHM) in radian, and θ is the Bragg angle (in degree), The mean size of TiO₂ nanoparticles was about 3 nm.

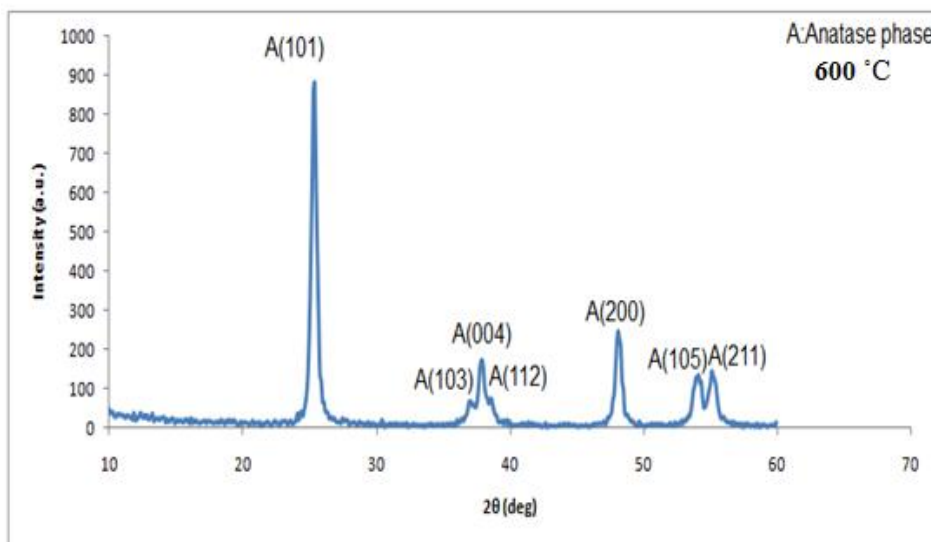


Figure (1) XRD pattern of TiO₂ anatase phase which have intensity at peaks (101), (004), (200), (105) and (211).

The UV-Vis. absorption spectrum of TiO₂ nanoparticles solution is shown in Figure (2) to have an absorption edge in the range of (330 -354) nm, indicating that TiO₂ colloidal solution obtained is anatase phase. The band gap energies (E_g) of the prepared TiO₂ nanoparticles (3.45 eV) as shown in Figure (3) which is larger than the value of (3.2 eV) for the bulk TiO₂. This can be explained because the band gap of the semiconductors has been found to be particle size dependent [26]. The band gap increases with decreasing particle size and the absorption edge is shifted to higher energy (blue shift) with decreasing particle size. Considering the blue shift of the absorption position from the bulk TiO₂, the absorption onset of the present samples can be assigned to the direct transition of electrons in the TiO₂ nanocrystals.

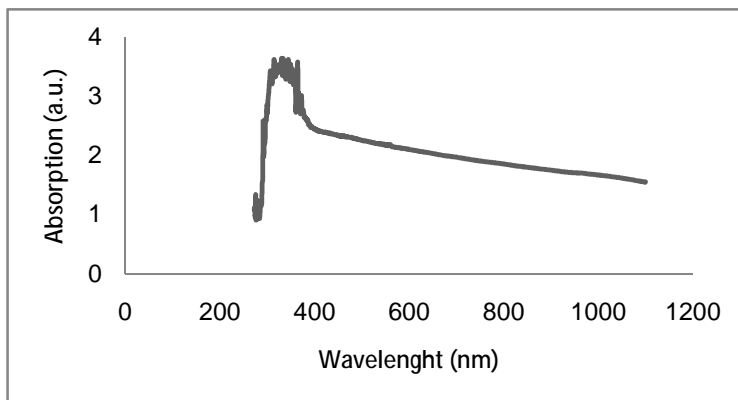


Figure (2) UV- Vis absorption spectrum of TiO₂ nanoparticles Via sol-gel method.

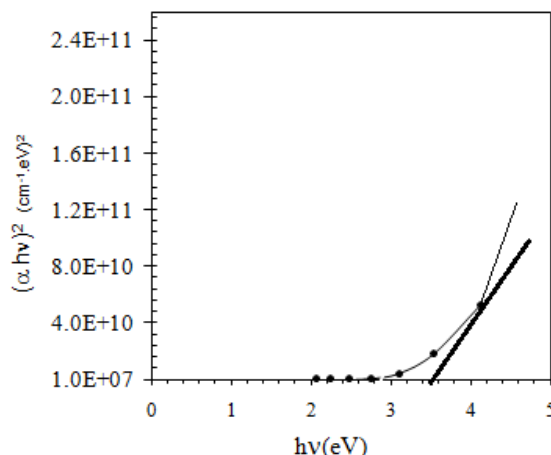


Figure (3) Band gap obtained by extrapolating the linear Portion of the $(\alpha h\nu)^2$ versus $(h\nu)$ curve.

Figure (4) shows Scanning Electron Microscope (SEM) images of TiO₂ samples derived TiO₂ nanoparticles in anatase phase calcinated at 600 °C for 2 hours. Clear nanostructures can be seen having grain size of 30 nm. It is clear that the TiO₂ nanoparticles seen by SEM image consist of number of crystallites. As compared with XRD results the grain size of TiO₂ nanoparticles characterized in XRD – Diffractometer by Debye-Sherrer equation are more smaller than the results observed by SEM. Scanning Electron Microscope (SEM) images shows that TiO₂ nanoparticles were agglomerated when the solution stringed in short time. In these Figures all dispersed particles were observed with size less than 50 nm.

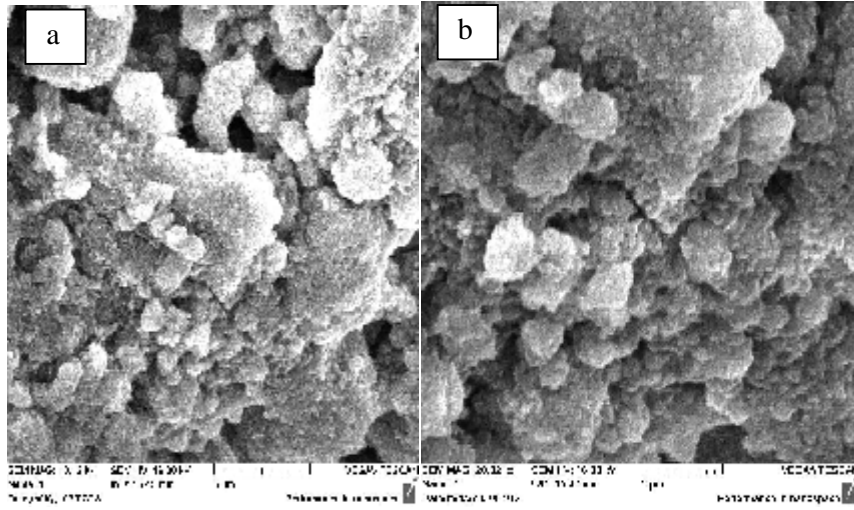


Figure (4) SEM micrographs showing morphologies of TiO₂ nanoparticles powder samples made in calcinations temperature 600 °C for 2 hours with two different magnifications (a) 5 μm and (b) 2 μm.

Antibacterial activity results

The antibacterial activity of TiO₂ for the TiO₂ finishing agents and treated bacteria were evaluated quantitatively according to Dow corning Shake Flask Test Method [27]. [The determines the reduction in the number of bacterial cells after placing the sample in the shaking flask for 24 h. Escherichia coli and Staphylococcus aureus, Gram-negative and Gram-positive bacteria respectively] were chosen as testing bacteria. 0.65 gm from TiO₂ nanoparticles in 100 ml of PBS (phosphate buffer saline) culture solution with cell concentration of $1-5 \times 10^6$ CFU /ml. The flask was then shaken at 250 r/min on a rotary shaker at 37 °C for 24 h. After shaking, 1 ml of testing solutions from, TiO₂ nanoparticles, PBS and two types of bacteria, gram negative and gram positive with different concentrations from (10^1) to (10^5) extracted, diluted and spread onto an agar plate (Nutrient Agar). After incubation at 37 °C for 24 h, the number of colonies formed on the agar plate was counted and the results were expressed as mean colony forming units per milliliter. Antibacterial efficacy was determined on the basis of duplicates test result. Percentage bacterial reduction was calculated according to equation 2:

$$R = (Z - A) / Z \times 100\% \text{ reduction [28]} \quad \dots (2)$$

Where R is the percentage of bacterial reduction; Z and A are the average number of live bacterial cell per milliliter. Figure (5) shows the images of antibacterial agents (TiO₂ nanoparticles) effect on two types of bacteria Escherichia coli and Staphylococcus aureus, respectively.

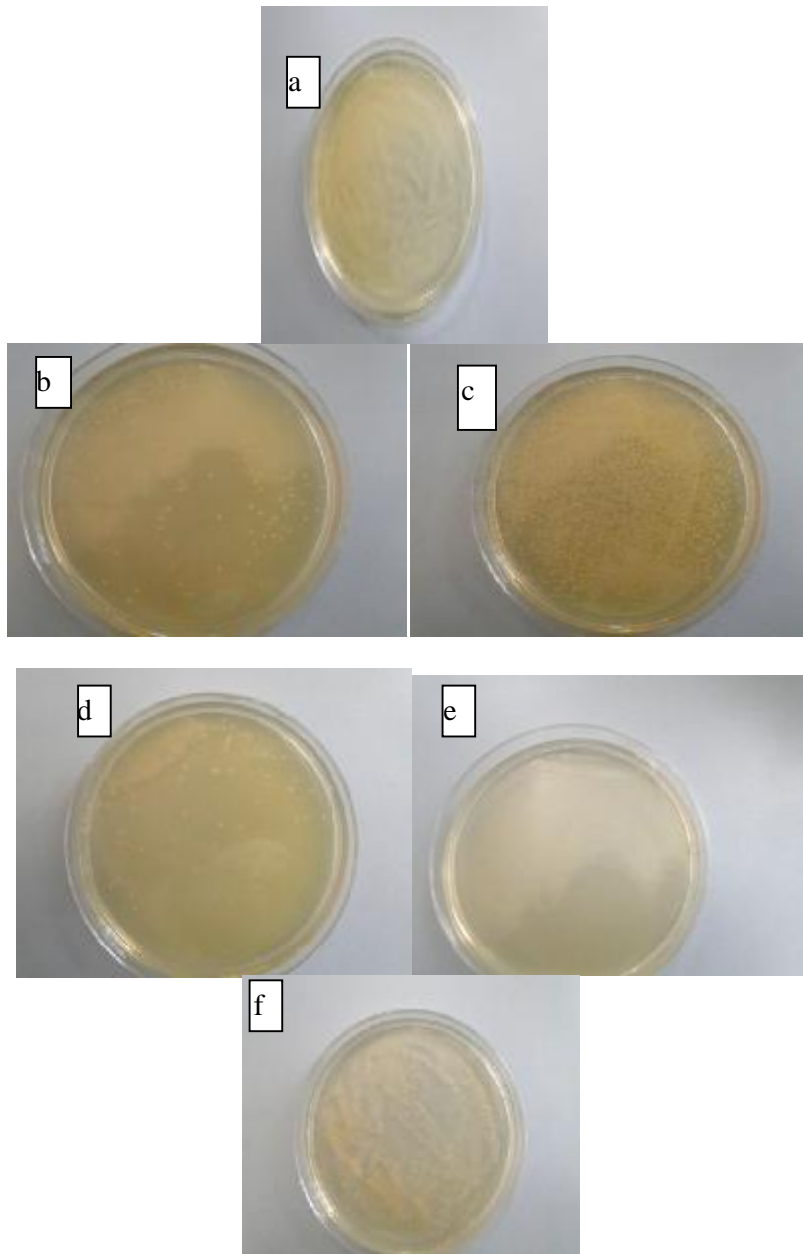


Figure (5) shows different images of different concentrations of TiO₂ nanoparticles and PBS with Escherichia coli and Staphylococcus aureus, respectively, (a) E-coli with PBS, (b) 10⁻³ TiO₂ nanoparticles, PBS and E- coli bacteria, (c) 10⁻⁵ TiO₂ nanoparticles, PBS and E- coli bacteria, (d) Staphy. Aurous with PBS: (e) 10⁻¹ TiO₂ nanoparticles, PBS and Staphy. Aurous bacteria, (f) 10⁻³ TiO₂ nanoparticles, PBS and Staphy. Aurous bacteria.

From the above results, it is generally agreed that the TiO₂ nanoparticles has stronger effect and more efficient on Staphylococcus aureus with 10⁻³ concentration than E-coli. While the efficient concentration of E-coli bacteria is 10⁻⁵. The reason may attribute to cell wall differences between the two distant bacteria, in which Escherichia coli has thinner and slack cell walls, while the results of Staphylococcus aureus showed that most sensitive bacteria against tested TiO₂ nanoparticles in comparison with E-coli bacteria. The mechanism of action of this nanoparticles on bacterial cell, inhibit protein or polysaccharides (Mucopolysaccharides) synthesis. But the mechanism of genetic materials by this material is not fully understood. Suggested that the inhibition of cell wall or plasma membrane. The merit of this explanation depends much on the molecular organization of these particles. The last these particles may affect cell division by modifying the cellular environment but induce damages through a direct action on the cell wall and plasma membrane which become weaker region which suspected that dividing cells.

CONCLUSIONS

The TiO₂ nanoparticles which was synthesized by sol-gel method is a viable material for antibacterial agents of gram negative and gram positive, *Escherichia coli* (E-coli) and Staphylococcus aureus, respectively. The TiO₂ was tetragonal anatase phase with 30 nm in size by Scanning Electron Microscope (SEM). The anatase phase was confirmed by X-ray diffraction pattern with sharp peak at 2θ= 25.39° [101] and 3.45eV energy band gap, Eg characterized by UV-Visible Spectrophotometer. The more efficient phase as antibacterial agent was anatase and TiO₂ nanoparticles were effective and sensitive with *Staphylococcus aureus* in 10⁻³ concentration more than *Escherichia coli* (E-coli) with 10⁻⁵ concentration.

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