The Antibacterial Activity of TiO_2 Nanoparticles

Saba A. Mahdy **Wasnaa H. Mohammed** **Hamsa Emad**
Biotechnology division, Applied Science department
University of Technology, Baghdad
saba.hadi@yahoo.com wasnaa_14@yahoo.com
Huda Abdul Kareem **Rana Shamel** **Suzan Mahdi**
Biotechnology division, Applied Science department
University of Technology, Baghdad

Abstract

The potent antimicrobial efficacy of nanoparticles compounds represents new solution to microbial challenges in many infectious diseases. In the present study TiO_2 nanoparticles which prepared by sol-gel method with 33nm size posses marked antibacterial effect on gram positive *Staphylococcus aureus* strain and gram negative *Escherichia coli* strain. The minimum inhibition concentration of TiO_2 nanoprticles was found to be 30 µg/ml for *S. aureus* and40µg/ml for *E. coli*, which was determined by broth dilution method with and without TiO_2 nanoparticles. The results were confirmed by agar diffusion assay.

Keywords: TiO2 nanoparticle, broth diffusion assay, Minimum inhibitory concentration, Antibacterial effect

الخلاصة

تمثل الفعالية الضد ميكروبة للمركبات النانوية النشطة بيولوجيا الحل الجديد للتحديات الميكروبية في العديد من الأمراض المعدية. في الدراسة الحالية تم تحضير مركب اوكسيد التيتانيوم النانوي بطريقة سول–جل بحجم 33 نانومتر والذي يمتلك تأثير مضاد ملحوظ لنمو البكتريا الموجبة لصبغة كرام المكورات العنقودية الذهبية والسالبة لصبغة كرام الإشريكية القولونية. كان التركيز التثبيط الأدنى لمركب اوكسيد التيتانيوم النانوي30 ميكروغرام / مل *Staphylococcus aureus* و40 ميكروغرام / مل *E. coli* والتي تم تحديدها بطريقة النمو في الوسط السائل مع وبدون اوكسيد التيتانيوم النانوي تم تأكيد النتائج وفقا لطريقة الانتشار في الوسط الصلب.

الكلمات المفتاحية: الجسيمات النانوية TiO2، مقايسة نشر المرق، والحد الأدنى من تركيز المثبطة، وتأثير مضاد للجراثيم

Introduction

Nano-materials are attracting a great deal of attention because of their potential for achieving specific processes and selectivity, especially in biological and pharmaceutical applications (Rammal *et. al*, 2002).

Titanium dioxide is occur a naturally as titanium oxide which has many properties such as high refractive index, Absorption of light, free toxicity, stability and less-cost production (Kamat *et. al*, 2002). Titanium dioxide nanoparticles these days have attracting a great deal of Interest because it have properties to achieve highly effect in biological, pharmaceutical applications, purification of environmental source, electronic system, cells of solar energy, photocatalysts, photo electrodes and sensors of gas beside American Food and Drug Administration (FDA) acceptance for using in food technology and drugs, cosmetics, paints pigment, ointments, and toothpaste (Awati *et. al*, 2002).

The activity of TiO_2 nanoparticles (1-100) nm in diameter is interesting to investigators because of specific characterization as size, shape, and structure of TiO_2 nanomaterials crystal, surface stability, the transferences between different phases of TiO_2 under stress and heat. Tio_2 Nano-Materials known as glamour of resent medical research due to its microbicidal effect to different diseases-causing organisms (Zallen *et. al*,2006). The bactericidal effect of TiO_2 nanoparticles on bacteria is extreme importance due to ability of pathogenic bacteria to enter in ecosystem food chain. (Zhang *et. al*, 2009).

The antibacterial activity of TiO_2 is due to the ability to activate free hydroxyl radicals (^{-}OH) by TiO_2 particles (Gutierrez *et. al*, 2010).The antimicrobial effect of TiO_2 against fungi and bacteria has been demonstrated (Lopez *et. al*, 2012) and communicating in modern research

The objective of recent study was to synthesis TiO_2 and use it to study the antibacterial activity on *Escherichia coli and Staphylococcus aureus* to anticipate information to improve new antimicrobial disinfecting solutions can be used as effective biofilm in food processing technology.

Materials and Methods

AChemicals

Muller Hinton media, Muller Hinton Agar was obtained from Hi-Media, Mumbai, India. Titanium (IV) isopropoxide and isopropanol was supplied from (sigma –aldrich).

B.Organisms

The microorganisms *E.coli, S. aureus* were grown in Muller Hinton broth aerobically at 37°C for 16 –18 h. A 10 µl loop transfer was performed and the strains were grown at 37 °C for another 24 h to achieve a population of 1×10^9 CFU. Serial tenfold dilution were performed in sterile 0.1% peptone water and inoculated into Muller Hinton broth so as to achieve target populations of 1×10^4 CFU and 1×10^8CFU, respectively.

C.Synthesis of TiO_2 nanoparticles

TiO_2 nanoparticles prepared by sol-gel methods. 100g of Titanium isopropoxide was mix with 200ml of iso-propanol for 5min stirring, mixture consist of (25.33ml) water and (127ml) iso-propanol was adding drop by drop to alkoxide solution, after stirred for 24 hours, the precipitate was drying at 100 °C in a hot aeration oven. Finally the sample calcined at 500 °C and 1000°C. The results determined by XRD, UV-Vis Spectrophotometer and SEM.

D. Antimicrobial Effect of TiO_2 in Liquid media

To study the bacterial concentration 1 mg/ml of TiO_2 nanoparticles sonicated for 5 minutes to sterilization and homogenous suspension under UV for 30 min. *E.coli and S. aureus* were added into Muller Hinton broth with various concentrations of TiO_2 nanoparticles (0 µg, 10 µg, 20 µg, 30 µg, 40 µg, 50 µg, 60 µg, 70 µg, 80 µg, 90 µg and 100 µg)/ml and incubated over night at 37°C, The optical density results to growth were reading at 600 nm.

E. The antimicrobial efficiency of TiO_2 nanoparticles by agar diffusion assay.

On Muller Hinton agar Media 100 µl of Bacterial culture was spared and six wells were made in each plate then filled with various concentrations of TiO_2 nanoparticles (0 %, 0.5%, 1%, 1.5%, 2% and 2.5%). The zone of inhibition measured by mm after 24 hours of incubation represented the antimicrobial effect of TiO_2 nanoparticles.

Results and Discussion

Several ways have been involved to take out a better synthesis of TiO_2 nanoparticles, free from toxic chemical, easily available, safe to handle and reduces the cost competitive (Sahoo *et. al*, 2007). In our study the synthesis of TiO_2 nanoparticles done by sol-gel method. Changing the color of solution after 24 hours indicate the formation of TiO_2 nanoparticles and the results of the UV-visible spectroscopy were indicated the synthesis of nanoparticles by apparent broadening beak at 450 nm presented in (Fig. 1).The nano size of titanium dioxide determined by SEM image (Fig. 2) with size 33nm these results conformed with XRD manner which

recorded by XRD-IND-MET (MDI/JADE7) TDXRD CSC diffractometer at Cu/K-alpha 1 radiation, TiO2 Nanoparticles Which intended with sol-gel method is fairly anatase structure and the crystal face is steady due to (110) the strongest peak in sharpness among to Diffraction peaks (002), (002), (200), (202), (020), (113), (311) and (220).

The grain size average of sample depending to Scherrer formula is 33nm. (Fig. 3) show X ray diffraction picture of sample.

E.coli and *S. aureus* are the most infectious agents of nosocomial diseases beside having broad spectrum antibiotics resistant because of excessive using of the antibiotics (Haghi *et. al*, 2012; Shiraishi *et. al*, 2008). Many studies issue the ability of using of metal oxide nanoparticles as alternative solution to antibiotics due to strong germicidal nature of it. The antimicrobial activity of TiO_2 nanoparticles on bacterial growth in liquid media are shown in (Fig. 4). In this experiment the strain of *E.coli* and *S. aureus* were inoculated in Muller Hinton medium with different concentrations of TiO_2 nanoparticles. The MIC values of TiO_2 nanoparticles was30 µg/ml, for *E.coli* and 40µg/ml for *S. aureus*. Basically the growth of *E.coli* and *S. aureus* inhibited compared to the control and the results inversely proportional with increasing the concentration of TiO_2 nanoparticles, these results assured with the agar diffusion methods which show remarked susceptibilities of *E.coli* and *S. aureus* to TiO_2 nanoparticles from the results of test which presented in Table1 and (Fig. 5). Show killing effect of sample on *S. aureus* in all concentration.

Several studies have believed the germicidal mechanisms of TiO_2 nanoparticles involving release of positively charge ions to reaction medium linked to (negative charges) thiol group (-SH) of the proteins on the cytoplasmic membrane(Jayaseelan *et al*, 2013). This reaction lead to capture the cell wall and increased permeability beside it cause deform the structure of cellular components such as DNA, ribosomes and cellular enzymes and finally death of microbial cell (Koseki *et. al*, 2009).

Conclusion

Titanium dioxide nanoparticales displayed good activity against all the indicator pathogens showing it potential broad spectrum antimicrobial activity. The impressive properties of TiO_2 nanoparticles open the prospect for seriously using as alternative to antibiotics, treat many infectious diseases and self-disinfecting in hospitals and food industries and ability to used it as biopreservative.

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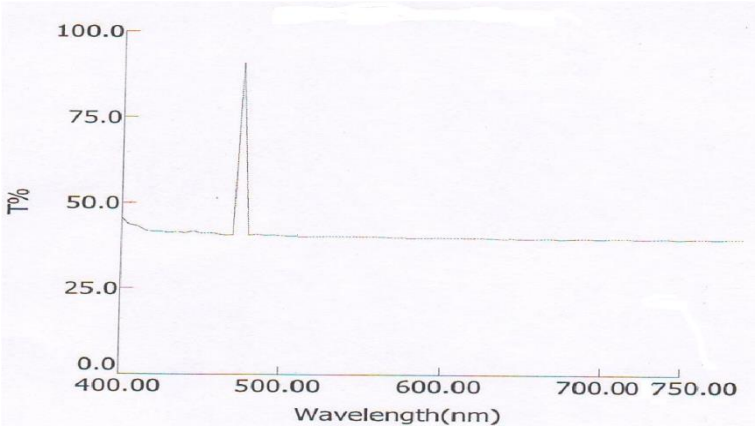

Fig.1. UV-Vis spectrophotometer indicate the formation of TiO_2 nanoparticles

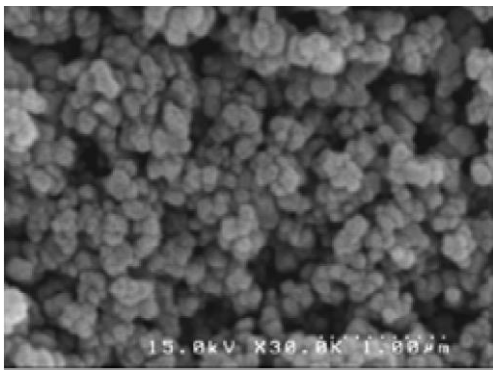

Fig.2. SEM images of TiO2 nanoparticles.

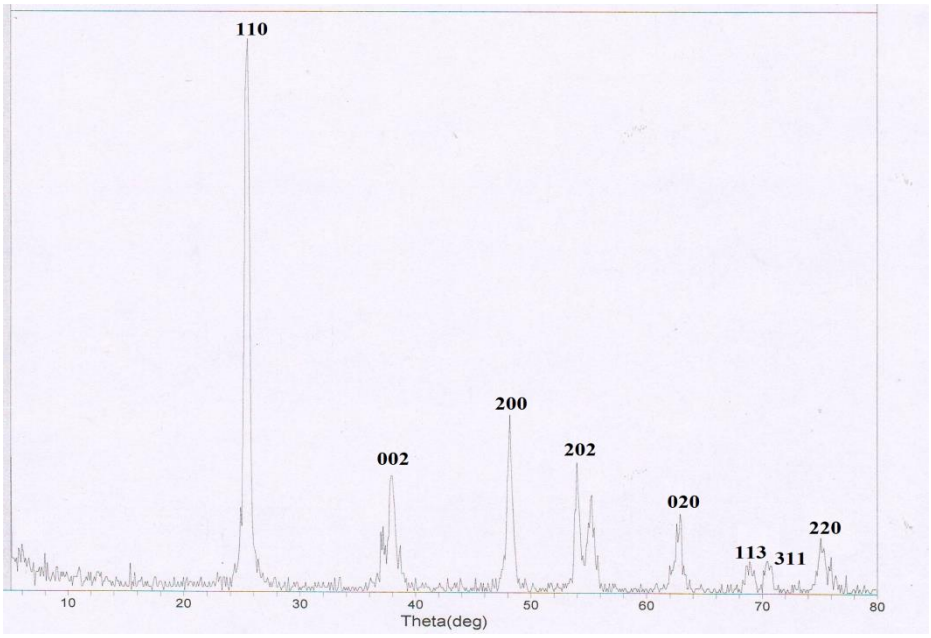

Fig.3. XRD of TiO_2 nanoparticles.

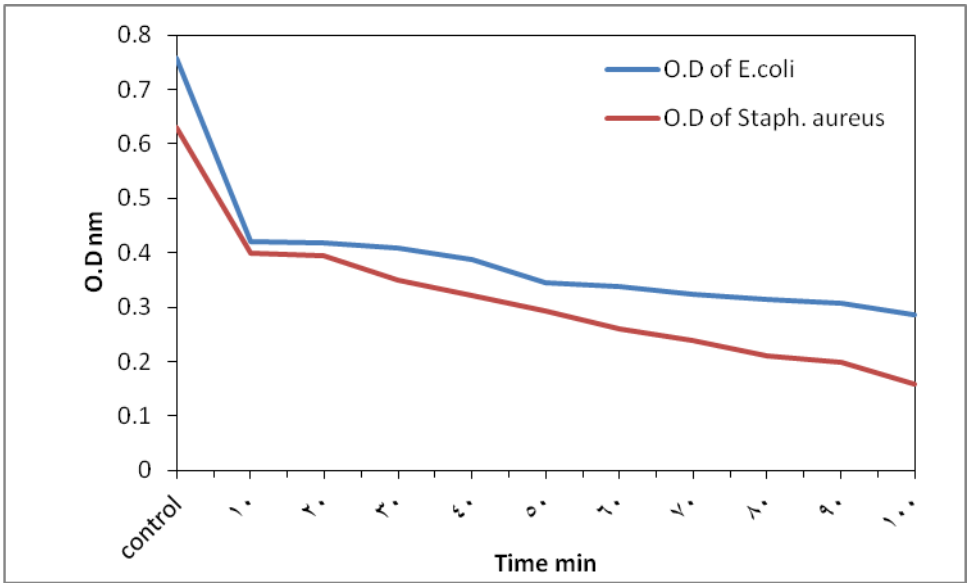

Fig.4. The inhibition effect of TiO_2 nanoparticles on pathogenic bacteria.

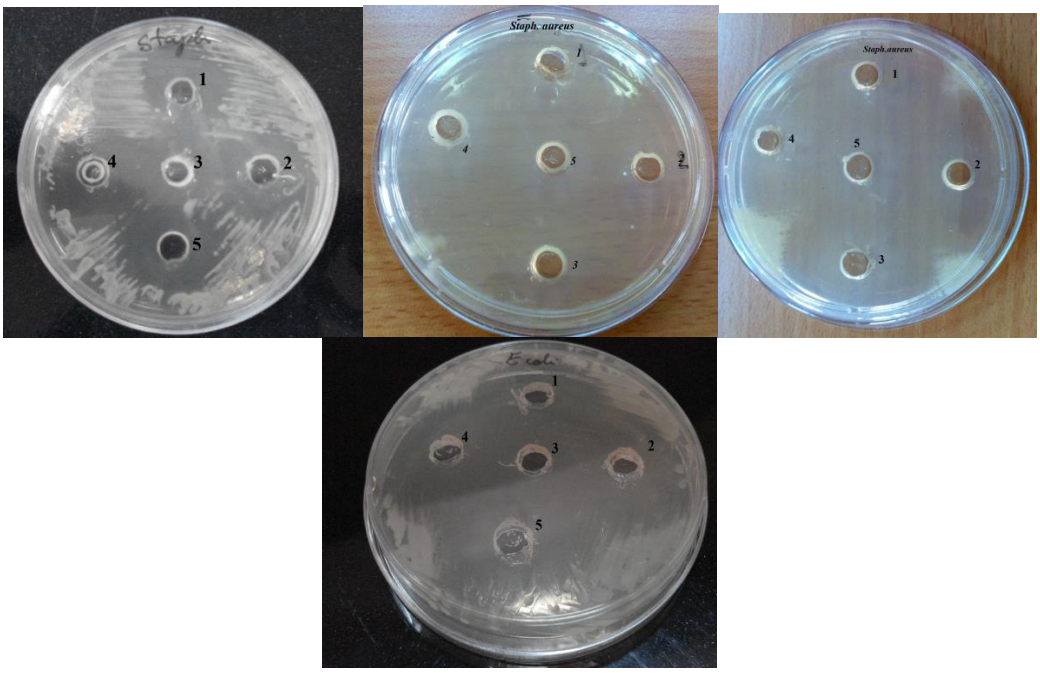

Fig.5. Antimicrobial Effect of TiO_2 in Agar Diffusion Method.

Table.1. Antimicrobial efficiency of TiO_2 nanoparticle against bacteria by agar diffusion assay.

No.	TiO_2 Conctration	*S. aureus*	*E.coli*
1	0.5mg/ml	no.growth	32mm
2	1mg/ml	no.growth	39mm
3	1.5mg/ml	no.growth	40mm
4	2mg/ml	no.growth	43mm
5	2.5mg/ml	no.growth	45mm