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ORIGINAL ARTICLE **OPEN ACCESS**

Gram- Negative and Gram- Positive Bacteria; Antibacterial activity of a clay-TiO_2 nanocomposits

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ABSTRACT
Nanotechnology has gained much public interest caused by the needs and applications of nanomaterials in many areas of human attempts such as industry, agriculture, business, medicine, public health among many others. In this research a new Titanium nanocomposite was synthesized and characterized. The nanocomposite, which synthesized is in proportion to the weight of Clay-TiO_2. The properties of this composition were looked at by the Fourier transform infrared spectroscopy (FT-IR), X-ray Diffraction (XRD) and scanning tuning microscopy (STM). Using lightweight aluminum and Titanium alloys in these industries has grown considerably. Anatase TiO_2 particles in aqueous solution by hydrolysis of Titanium is prop oxide in an acidic environment. Since Titanium's compounds have biological properties, the anti-bacterial properties were studied on four Gram-negative and Gram-positive batteries, E. coli, Pseudomonas aeruginosa, Micrococcus, Staphylococcus aureus. The present investigation was aimed to production a new nanocomposite of TiO_2 and determination of the antibacterial activity of this nanocomposite toward E. coli and Pseudomonas aeruginosa as Gram-negative bacteria. Micrococcus, Staphylococcus aureus as Gram-positive bacteria in laboratory condition.
Keywords: *Clay-TiO_2, Microbiological studies, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus.*

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INTRODUCTION

Nanotechnology is the ability to work on a scale of about 1–100 nm to understand, create, characterize and use material structures, devices and systems with new properties derived from their nanostructures. All biological and man-made systems have the first level an organization at the nanoscale. By using nanotechnology techniques, it is possible to assemble molecules into objects, along several length scales, and to disassemble objects into molecules, as nature already does [1].

Two building strategies are used in nanotechnology: a "top-down" approach and the "bottom-up" approach. Commercial scale producing nanomaterials involves the "top-down" approach, in which nanometric structures are got by size, cut bulk materials, by using milling, nanolithography, or precision engineering. Smaller sizes, meaning a bigger surface area, desirable for several purposes [2, 3].

The newer "bottom-up" approach, on the other hand, allows nanostructures to be built from individual atoms or molecules capable of self-assembling [4, 5]. Many materials which were considered to be safe develop toxicity at nanosize ranges which is mainly related about the raised specific surface area and high reactivity of nanosize materials [6, 7]. A larger surface area as for nanocomposites ensures a higher range probable interaction with bio-organics present on the workable cell surface[8]. The considerable antimicrobial activities of nanomaterials such as nano TiO_2 and their selective toxicity to biological systems suggest their potential application as therapeutics, diagnostics, surgical devices and Nanomedicine based antimicrobial agents [9, 10].

The advantages of using these inorganic oxide nanocomposites as antimicrobial agents are their greater effectiveness on resistant strains of microbial pathogens, less toxicity and heat resistance [11, 12]. Also, they provide mineral parts essential to human cells and even small amounts of them show strong activity [13, 14]. These metal oxide nanocomposites have many significant features such as chemical and physical stability, antibacterial activity, intensive ultraviolet and infrared adsorption with a broad range of applications as semiconductors, sensors, transparent electrodes, solar cells, etc [15]. The present investigation was aimed to production a new nanocomposite of TiO_2 and determination of the antibacterial activity of this nanocomposite toward E. coli and Pseudomonas aeruginosa as Gram-negative bacteria. Micrococcus, Staphylococcus aureus as Gram-positive bacteria in laboratory condition [16-18].

MATERIAL AND METHODS

In this study, the clay powder (with purity higher than90%) and Titanium oxide nanopowder with an average grain size 21 nm (with a purity of 99.9%) of which approximately 80% and 20% rutile Anatase it is with the weight ratio was used. All solvents were used without purification. Compounds purchased from Aldrich and the Merck Company purities were used without purification. Vibrational spectra of complexes by Fourier transform infrared spectroscopy (FT-IR) Bruker Tensor 27 machine made in Germany and the model 420 using KBr disks were taken. Phases in the samples for the detection of X-ray diffraction (XRD) device Siemens D500 Diffract meter model under voltage 30kV and 25 mA current was used. In all phases of Cu-Kαradiation with a wavelength of 1.5404 Å was used. The surface morphology of product was characterized by using a scanning tunneling microscopy (NAMA-STM Model SS2, NATSYCO, Iran). UV-Vis absorption spectra of solutions were recorded by a Perkin-Elmer double beam scanning spectrometer, Model Lambda 19.

PRWPARATION OF CALY-TiO_2 NANOPARTICLE

0.5 g TiO_2 was taken to the beaker (50ml) and in solved in 50ml of water. Then this part of the beaker was added to another beaker that is including 5 g clay. The whole solution was mixed; finally the compound was precipitated and separated. The product, dried at room temperate for 3 hours. Then the nanocomposite was taken to the electric furnace at 1850C for 5 h until to calcination.

CHARACTERIZATION OF NANOCOMPOSITE

The new nanocomposite were characterized by Fourier transform infrared spectroscopy (FT-IR), X-ray Diffraction (XRD) and scanning tuning microscopy (STM), techniques. Vibrational spectrum shows the bands of parts such as TiO_2 (Figure 1, Table 1). The X-ray Diffraction pattern showed formatting a nanocomposite. The pattern was similar to other published nanocomposites (Figure 2) Scanning tuning microscopy (STM), pictures shown composting nanocomposites, especially different morphology and texture could be found in pictures that in which shown the Magnification a) 1000 times, b) 5,000 times, c) 10,000 times, d) 30,000times, e) 50,000times, f) shows the particle size in ultrasonic waves. (Figure 3)

TABLE 1. Antibacterial Activity of this new clay-TiO_2 nanocomposite.

Micrococcus	*Staphylococcus aureus*	E. coli	*Pseudomonas aeruginosa*
7 mm	-	7 mm	-

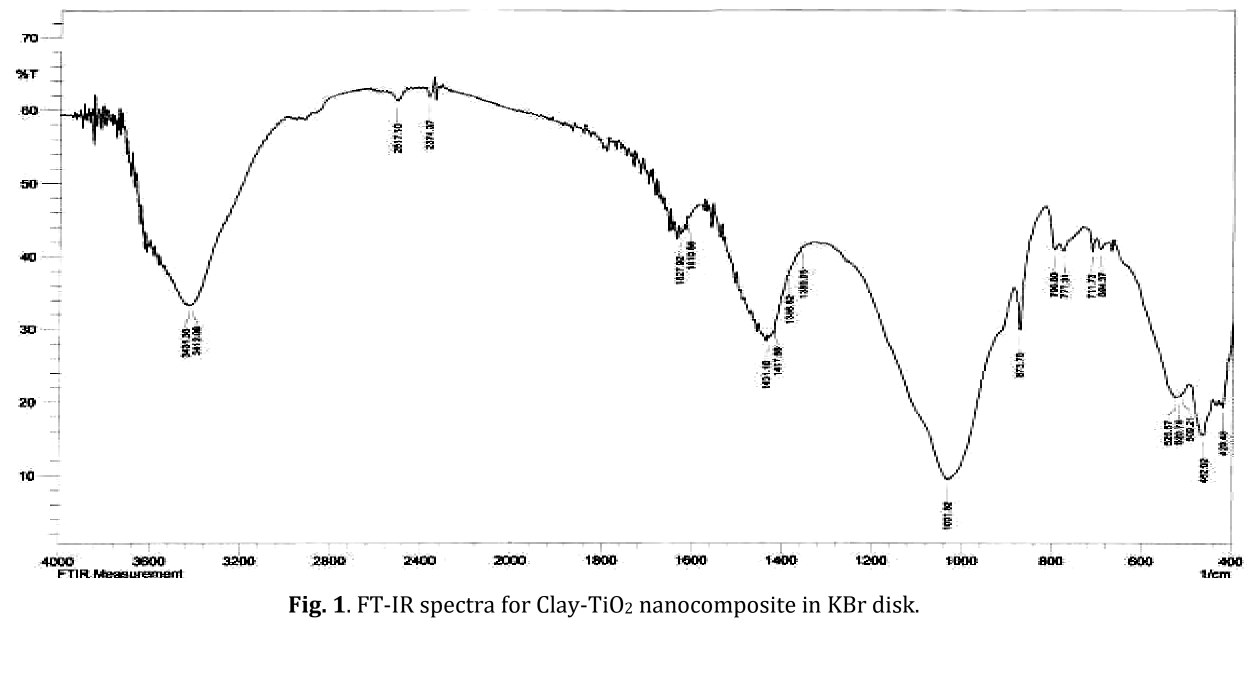


Fig. 1. FT-IR spectra for Clay-TiO_2 nanocomposite in KBr disk.

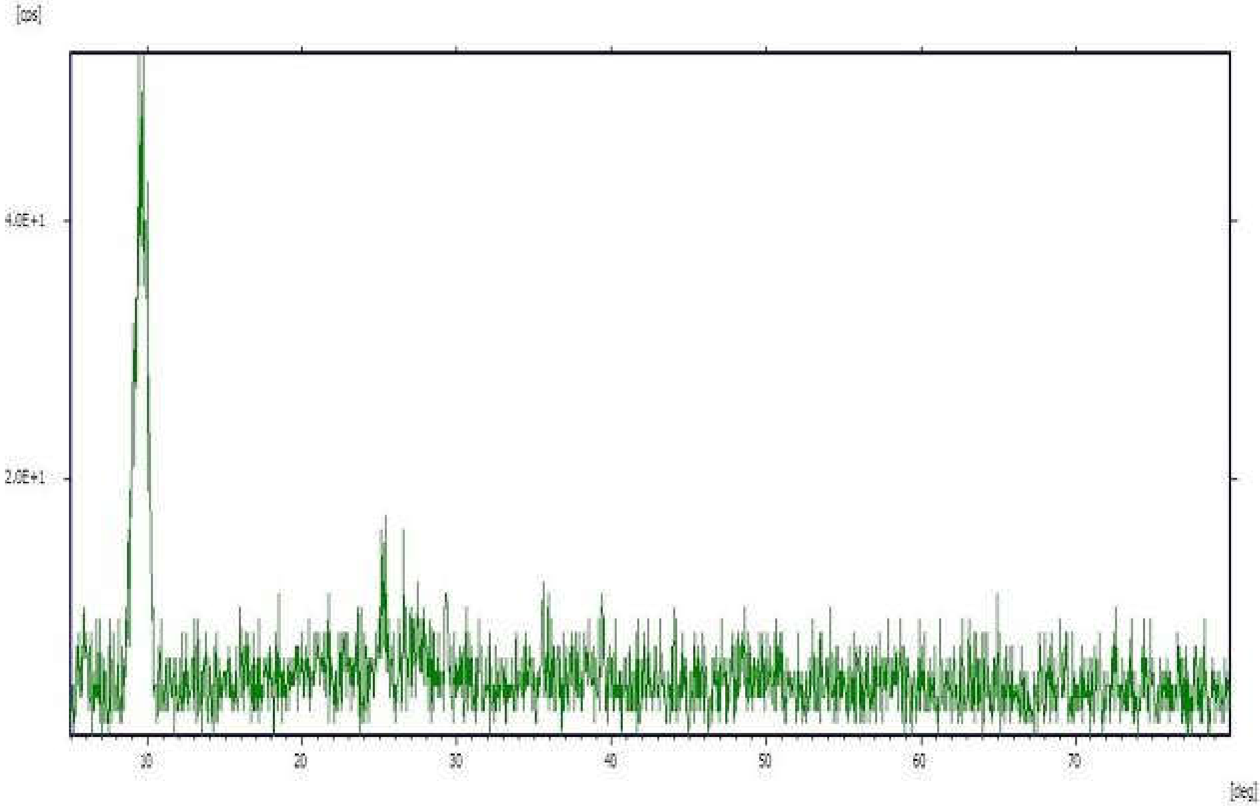


Fig. 2. X-ray Diffraction pattern of Clay-TiO_2 nanocomposite

RESULTS AND DISCUSSIONS

BACTERIAL CALTURES AND TEST OF ANTIBACTERIAL ACTIVITIES

Films with antimicrobial activity could help control the growth of pathogenic and spoilage microorganisms. An antimicrobial nanocomposite film is desirable because of its acceptable structural integrity and barrier properties imparted by the nanocomposite matrix, and the antimicrobial properties contributed by the natural antimicrobial agents [19].

Materials in the nanoscale range have a higher surface-to-volume ratio when compared with their microscale counterparts. This allows nanomaterials to be able to attach more copies of biological molecules, which confers greater efficiency [20]. Four types of bacteria Micrococcus, Staphylococcus aureus as a Gram-positive bacterium, and E. coli and *Pseudomonas aeruginosa* as a Gram-negative bacterium, were used as model bacteria in this study. Luria broth (LB) and agar were used as sources for culturing E. coli at 37 °C on a rotary platform in an incubator. In vitro activity test was carried out using the growth inhibitory zone (well method) [21-24].

VIBRATIONAL SPECTRA

Fourier transform infrared spectroscopy (FT-IR) absorption was used to check the characteristic bands of the synthesized powder. Similar spectra were observed for other nanocomposits. The bands at 450-900 cm^{-1} were related on Ti-O bonds [25, 26] (Figure 1, Table 2). The spectrum shows two strong IR absorption bands at ~3154.39 and 1409.33cm ^{1} which are characteristic of H–O–H bending of the H2O molecules showing the presence hydroxyl groups in the as prepared sample.

TABLE 2. Vibrational spectra for title compounds.

Vibrational bond	Intensity	Wavelength cm^{-1}
ν (Ti-O)	s	462
ν (Ti-O)	m	520
ν (Si-O)	s	873
ν (O-H)	m	3431

X-RAY DIFFRACTION ANALYSIS

The overall shape of the pattern and angular peaks shows the nano properties of a nanocomposite. The characteristic parts could be found in the X-ray Diffraction pattern such as absorption 2θ = 25.50, related totheTiO_2 andpeak at 2θ = 9.50, related to Clay.

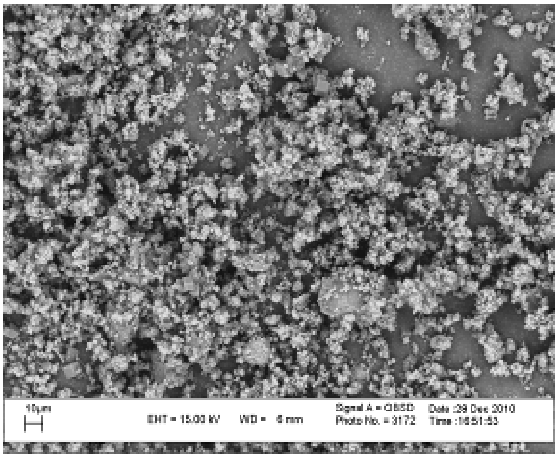
X-ray Diffraction spectra using the approximate size of the particles in the nanocomposite can be calculated. When particles are smaller than 100 nm X-ray Diffraction peaks are much wider than this factor can be used to estimate the size of then a particle. Shrr equation used for this purpose:

$$D=0.9\ \lambda\ /\ B\ \mathrm{Cos}\theta$$

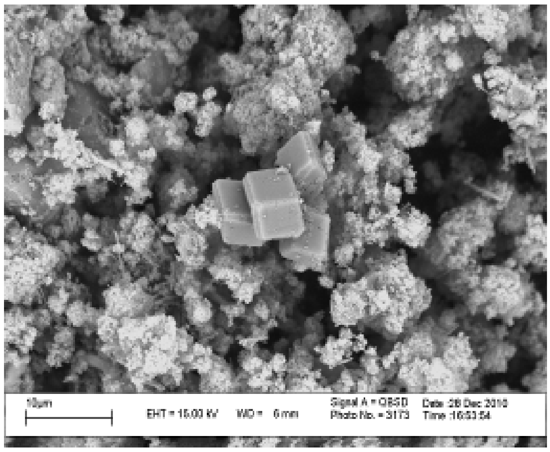
In the above equation D in terms of particle diameter Å, B corresponds to the width of the strongest peak at half height in radians, and θ is the angle at which the peak appears. X-ray Diffraction analysis shows the synthesized nanocomposites. The X-ray Diffraction pattern of nanocomposite Clay-TiO_2 in scattering observed and nano TiO_2 sharp peak appeared at 2Θ=9.50-10 is clearly seen in all samples.

SCANINIG TUNING MICROSCOPY (STM), PICTURES

Scanning tuning microscope image of Clay-TiO_2 nanocomposite with different magnification (a) 1000 times, b) 5,000 times, c) 10,000 times, d) 30,000times, e) 50,000times, f) shows the particle size in ultrasonic waves) for 5 hours at 185° Chas shown calcination. (Figure 3). Scanning tuning microscopic observations of the Clay-TiO_2 nanocomposite show that the powder particles due to heat released during calculation of the flexible and a raise in average particle size and morphology is irregular. Due to the heat released during calcination irregular particles are particles in different forms are observed. With increasing calcination time to 8 hours of powder particles due to higher heat release, the grain size was larger and thus reducing the distance between the atoms and consequently the particles are too large (Figure 3).



A



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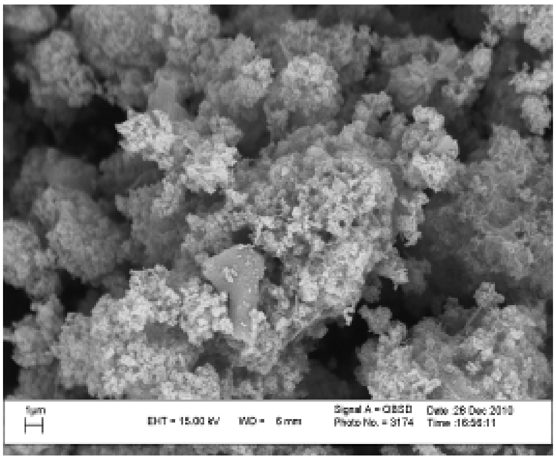
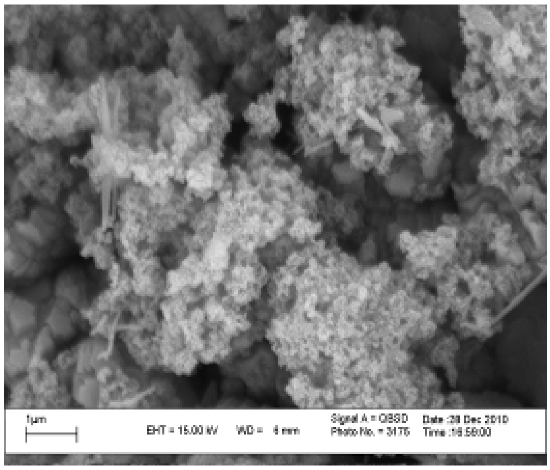
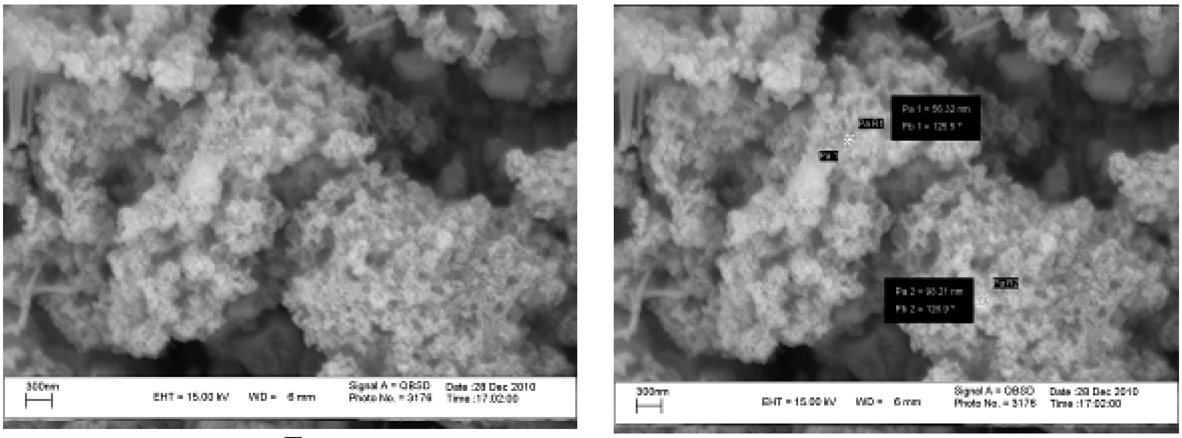




C D



E F

Fig. 3. Clay-TiO_2 nanocomposite morphology with Magnification a) 1000 times, b) 5,000 times, c) 10,000 times, d) 30,000 times, e) 50,000 times, f) shows the particle size in ultrasonic waves.

ANTIBACTERIAL ACTIVITY

The potency of parts was determined against the two Gram-positive bacteria and against the tow Gram-negative bacteria. Microorganisms (got from enrichment cultures of the microorganisms in 1 mL Muller-Hinton broth incubated at 37°C for 3 h were cultured on Muller-Hinton agar medium.

The inhibitory activity was compared with that of standard antibiotics, such as gentamicin (10 μg). After drilling wells in the medium using a 6 mm cork borer, 100 μL of solution from different compounds were poured into each well. The plates were incubated at 37°C overnight. The diameter of the inhibition zone was measured as precisely as possible. Each test was carried out in triplicate, and the average was calculated for inhibition zone diameters. A blank containing only DMSO showed no inhibition in a preliminary test. The macro dilution broth susceptibility assay was used for evaluating little inhibitory concentration (MIC).

The use 12 test tubes are required by the macro dilution method. By including 1 mL Muller-Hinton broth in each test and then adding 1 mL extract with concentration 100 mg/mL in the first tube, we made a serial dilution of this extract from the first tube to the last tube. Bacterial suspensions were ready to match the turbidity of 0.5 McFarland turbidity standards. Matching this turbidity provided bacterial inoculums concentration of 1.5×108 cfu/mL. Then 1 mL of bacterial suspension was added to each test tube. After incubation at 37°C for 24 h, the last tube was determined as the little inhibitory concentration (MIC) without turbidity (Table 1).

In recent years, cutting the structural weight of the factor considered by manufacturers, especially in aerospace and automobile industries, therefore, the use, lightweight aluminum and Titanium alloys in these industries has grown considerably. In this study, a novel and simple method were developed for the synthesis of highly nanostructured, Anatase TiO_2 particles in aqueous solution by hydrolysis of Titanium is prop oxide in an acidic environment. The results of characterizing the TiO_2 nanocomposite by UV-vis absorption spectroscopy, X-ray Diffraction (XRD), scanning tuning microscope showed that fairly uniform sized nanocomposite of 10-15 nm diameter with sphere-shaped Anatase form were getting. Results showed good inhibition on two types of bacteria, E. coli and Micrococcus, even under room light. A decrease in the survival ratio of E. coli and a fast killing effect were observed. Based on the X-ray Diffraction patterns, the peaks related on the calcination time can be cut TiO_2 (Anatase and Rvtayl) attributed. The effect of the compounds formed between the metallic pattern and the diffraction peak corresponding to the samples to be seen. In surveys taken from the IR spectra of Ti-O bonds were in the range of (450 cm^{-1}-900 cm^{-1}).

CONCLUSION

A new Titanium nanocomposite was synthesized and characterized. This compound, which synthesized is in proportion to the weight of Clay-TiO_2. The properties of this arrangement were observed by the Fourier transform infrared spectroscopy (FT-IR), X-ray Diffraction (XRD) and scanning tuning microscopy (STM). So, these compounds have biological properties, the anti-bacterial properties were studied on four Gram-negative and Gram-positive batteries, E.coli, Pseudomonas aeruginosa, Micrococcus, Staphylococcus aureus. Research results show that with decreasing particle size to nanometer amplifier and nanocomposite construction phase to ensure proper mixing amplifier Moreover, the strength, stiffness and raises flexibility. In recent years, cutting the structural weight is one of the most important factors considered by manufacturers; therefore, the use, lightweight aluminum and Titanium alloys in these industries have grown considerably.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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