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Preparation and Characterization of TiO_2 Nanoparticles and its Applications as Antibacterial Agents

Abstract- *TiO_2NP's were synthesis by sol-gel method at different calcination temperatures. The prepared TiO_2NP's were characterized by X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM), and UV-Vis spectrophotometer. The antimicrobial activities of the prepared TiO_2NP's were investigative for two kinds of bacteria, (Gram-negative and Gram-positive bacterium. XRD results shows that the type of the TiO2 structure was anatase (A) at low temperatures, rutile (R) with rise of calcination temperature (Tc) into 800 °C and mixed phases .SEM demonstrated that the size of nanoparticles seems larger and the accumulations appears clearly with raise calcinations temperature. The optical properties measured by the UV-Vis. Spectroscopy to compute the energy band gap for all phases around (3.75- 3.5 eV) for anatase and (3.4eV) for rutile. TiO_2 nanoparticles in both phases showed excellent antibacterial activity against two representative bacteria, Pseudomonas aeruginosa and Staphylococcus aurous.*

Keywords- *TiO_2 nanoparticles; sol-gel method; antibacterial activity.*

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1. Introduction

In the present century, Nanotechnology has gained great attention because of its ability in allowing for the manipulation of materials and metals into nanosized that causes a drastic change in properties [1]. Nanotechnology has enable to provide a new class of atomic scale materials such as Nano-sized metal oxide catalysts that have high activity in the degradation of a broad scale of organic and inorganic pollutions in water and air [2] according to their high reactivity, large specific surface area, greater selectivity, and superior durability [3]. Lastly many natural materials and manufacture nanostructure has been appear to has perfect antimicrobial properties. Among the metal oxide catalysts, Titanium dioxide (TiO_2) has become the prototype material. TiO_2 is a good material for the convert's harmful material into harmless of many pollutants [4] such as decolonization of dyeing waste water, air filtration and water and poison gas detection such as of NOx, SOx, also to early detection of viruses, and cancer cells [5]. The aim of the present study is to synthesize anatase TiO_2 by sol-gel method and to examine its antibacterial properties.

2. Experimental Part

I. Prepare of TiO_2NP's

All chemicals are of analytical grade, 20ml of TiCl4 (99.99%, BDH, England) was added drop slowly to 200 ml of absolute ethanol CH3CH2OH (99.99%, GCC, U.K) at room temperature (RT). Interaction was carried out in the smoke hood because a big product from Cl2 plus HCl that were exhausted through reaction process. It was obtained with a light yellow colour and Gelemattin solution to form a solution, and the pH of the solvent at range of (1-2).
Then, the obtained solvents were vaporized at 80 °C to become a dry-gel were got for 1.5 h in tube furnace with various calcinations temperatures (500, 800, and 1000) °C, to form TiO2 Nano-powders.

II. Measurements

The fabrication *TiO_2NP's* were measured of the structure phase by XRD-6000 2kw kind at RT, working voltage at 40 kV and 30 mA, Cu 1.54060 A[7]. Surface morphology was studied by SEM (Model TE-SCAN VEGA3 SB).The optical properties investigated by UV-Vis spectroscopy.

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III. Antibacterial Activity Test

A strain of gram- negative and positive of bacteria type Pseudomonas aeruginosa and Staphylococcus aurous were chosen respectively, as the model bacterial contaminant for this study. All bacteria were cultured overnight at 37 °C in nutrient agar (NA). The bio- activity of TiO_2 NP's were tested quantitatively corresponding to Dow corning Shake Flask Test Method. Bacterial inactivation was evaluated using TiO_2 solution added at five concentrations (1, 2.5, 5, 7.5, and 10) mg/ml and sterilized by autoclave for 15 min at 121°C and 15 Psi, then 1mL of the diluted cell suspensions with concentration of (1x107 CFU/ ml) was added. After that, the mixture was shaken (150 rpm) on a rotary shaker at 37°C for 24h. An overnight period, the suspension was sequentially diluted over an overnight period 10 times in normal saline water (100 µl distributed over a solid nutrient agar medium) using diffusion plate technique. The dishes were incubated overnight at 37 ° C. The number of living cell in the plates was then calculated by multiplying the number of colonies with the dilution factor and comparing it with the control, which represents the suspension of the bacteria only don't used TiO2, and then reduce the percentage was calculated according to the initial number given by the below formla [6]:

Number of Colonies [(CFU)/ml] = (Number of colonies for each dilution)* (dilution 103 factor)* sample volume (1)

Also, the percentage reductions of bacteria are calculate by bactericidal rate K [6]:

$$K = \frac{(A-B)}{A} X100\% \quad (2)$$

Where: A and B represent the no. of bacteria colonies corresponding to the control samples (without TiO_2) and sample with TiO_2.

3. Result and Discussion

I.XRD results

The phase composition and crystallite sizes of the synthesized TiO_2 nano-powders were explained by XRD analysis. Fig.1 refers the XRD structures of TiO_2 NP's gained by chemical reaction technique and calcined at various temperatures. The grain size was estimated by Scherrer's formala[7]:

$$D=K\lambda/(\beta\cos\theta)\ \% \quad (3)$$

Where: k is constant that up on the physical properties and shapes of the matter, λ is the X-ray wavelength, β is the FWHM of the maximum intensity and θ is the Bragg's angle. The (R/A) ratio was determined from the following equation [8]:

$$w_R = {}^{I_R}/_{I_O} = w_R = \frac{I_R}{(0.88I_A+I_R)}\% \quad (4)$$

Where WR is the R weight fraction in percent, IA and IR are integrated diffraction peak intensity from A (101) and R (110), respectively. Io is the total integrated (101) and (110) peak intensity. Table (1) illustrate the influence of calcination temperatures on the practical and structural characterization of TiO2 NP's.

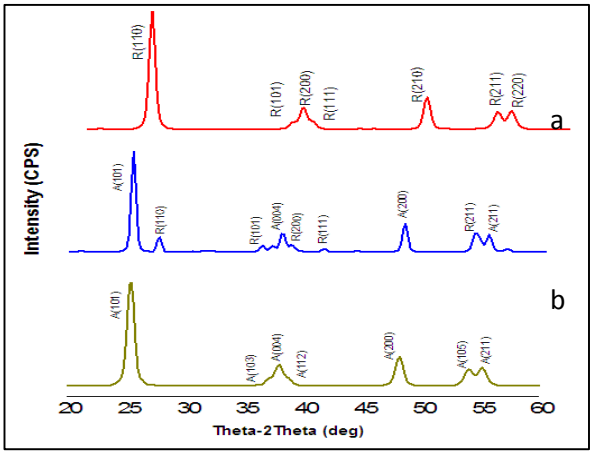


Figure 1: The XRD structure of TiO2 NP's at various calcinations temperatures: (a) 500, (b) 800, and (c) 1000 ◦C.

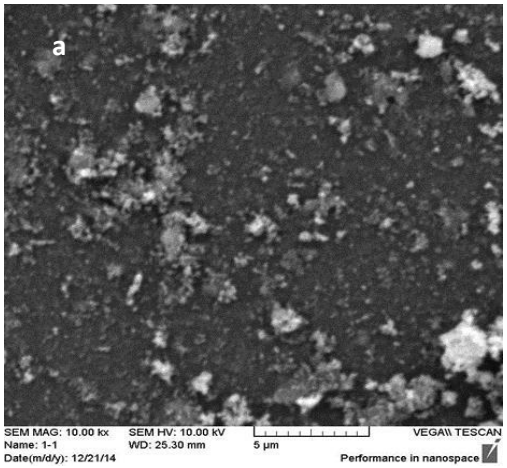
From the XRD patterns appears that the structure of TiO2 was anatase (A) at calcinations temperatures at 500 °C, rutile (R) at 1000 °C and hybrid phase at calcinations temperature of 800 °C. The A phase was represented at 2θ values of 55.101°, 53.901° 48.201°, 38.101° and 25.401° corresponds to the crystal planes of (105) ,(200), (004) and (101) respectively. Also, appears the R phase at 2θ values of 27.501°, 36.101°, 41.301°, 44.201° and 55.501° corresponds to the crystal planes of (110), (101), (200), (111), (210), (211), and (220) respectively. At a temperature of 800 ° C, very prominent R phase is high intensity. In addition, the phase components of this catalyst at this are 24.48% for rutile and 75.62% for anatase. When the size of crystals increases with the increase in the temperature of calcification because of the conglomeration of particles, the peak of the diffraction is narrower as in TiO_2 calcined at 1000 °C.

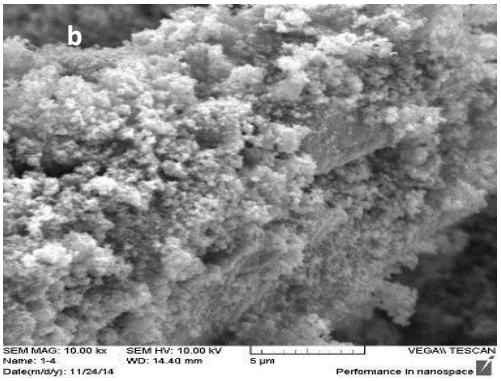
Table 1: Illustrate the influence of calcination temperatures on the practical and structural characterization of TiO2 NP's.

Calcination temp. (°C)	Phase[a]	2θ (Deg.)	β(FWHM) (Deg.)	Grain sized[b] (nm)	W_R %
500	A	25.4005	0.6209	12.5	0
		48.1266	0.6902	12.45	
		37.9087	0.8796	11	
800	A	25.4201	0.48020	16.97	24.48
	R	48.1525	0.50860	17.12	
		54.1737	0.82330	10.85	
1000	R	27.5531	0.50620	16.71	100
		54.4349	0.50910	16.43	
		36.1993	0.48250	18.51	

II. SEM results

Fig.2 shows morphological SEM images of TiO_2NP's synthesised with various calcinations temperature .From Fig. 2a seems TiO_2 nanoparticles calcined at 500 °C has non-uniform spherical porous shape with tiny particles approximately the average particle size of less than 10 nm. While, at the calcinaed temperature raises the particles size falls larger and the agglomeration fall more clear. When increasing temperature more than 800", and 1000 °C, TiO_2 NP's appears unregular form because of this agglomeration of start atoms with a raise of grain size. This behaviour coincident with XRD tests that explained the particles diameters of the anatase phase minimal than rutile phase due to aggregation of NP's with the increase of calcination temperature.





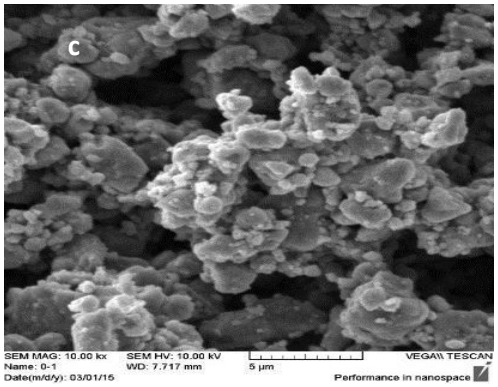


Figure 2: SEM images of TiO_2 NP's calcined with various calcination temperatures: (a) 500, (b) 800, and (c) 1000 ◦C.

III. UV-Visible Spectrophotometer

The transmittance of TiO_2 nanoparticles deposited coatings calcined at various temperatures seen in Figure 3. For the all TiO_2 coated films were analysed, the average transmittance is about 80-90% in the visible region of the spectra (400-700) nm. We are observed that the optical transmittance a little decrease with raising the calcinations temperature. These changes of the transmittances are associated with the increase the average particle size of the prepared TiO_2 coated films. Wang et al. [9] stated that the size of the particles proportional with the light transmission, increasing crystal size reduces light transmission. With the increase of the calcination temperature, the absorption edge wavelength shows a slight pseudo "red shift" also the transmittance of the coated layer will decrease this is because of increased of particles size with increase calcination temperature which lead to high peak absorbance with minimum energy band gap. Agglomeration of the crystallites at the highest temperature are responsible for this behaviour according to the results obtained by [10]. Also, the changes in the transmittance spectra and absorption edge wavelength of TiO2 coated surface calcined at different temperatures are

attributed to differences in surface thickness of nanoparticles on the deposited slides, surface microstructure, carrier concentration, and absorption of light.

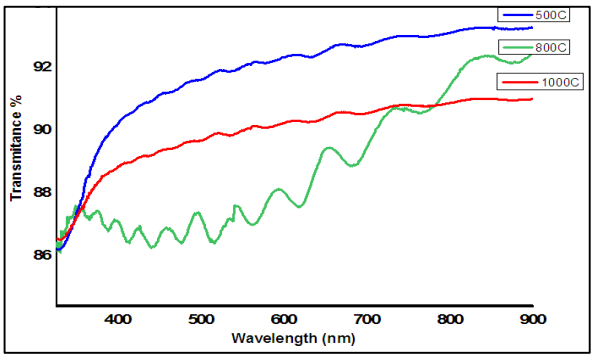
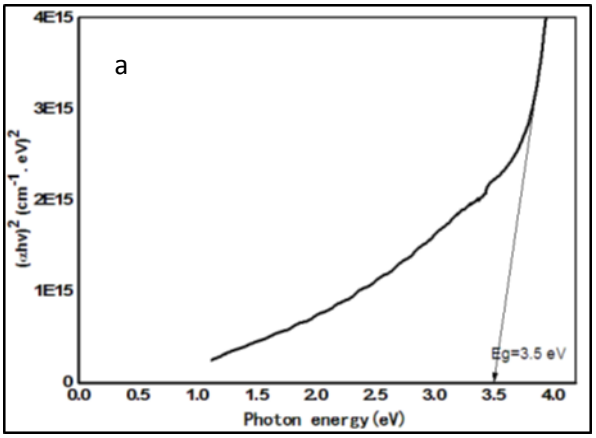
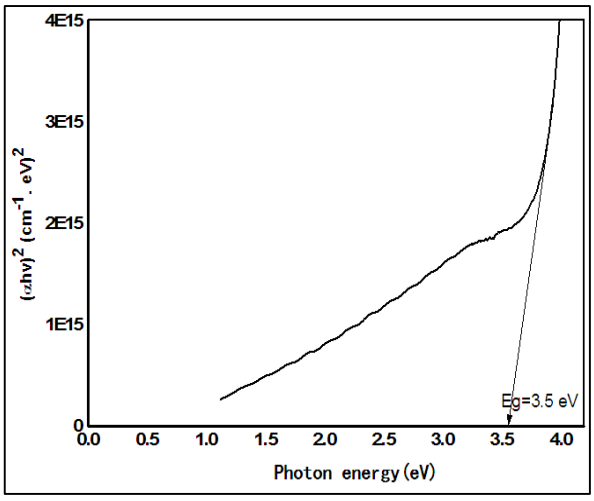


Figure 3: UV-VIS. Spectra with energy band gab of TiO_2 NP's at various temperatures

The energy band gap (Eg) means the forbidden gap(direct transition) between the top edge of the valence band (V.B) and the bottom edge of the conduction band(C.B). The sketch $(\alpha h\upsilon)^2$ as a against of photon energy is shown in figure 4. It is obtained from intercept of the extrapolated linear part of the curve with the x- axis.





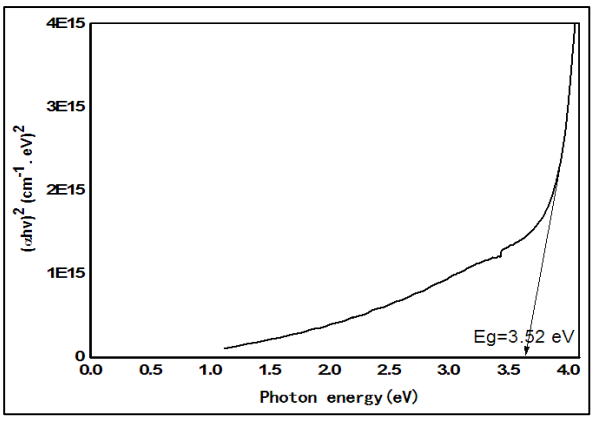
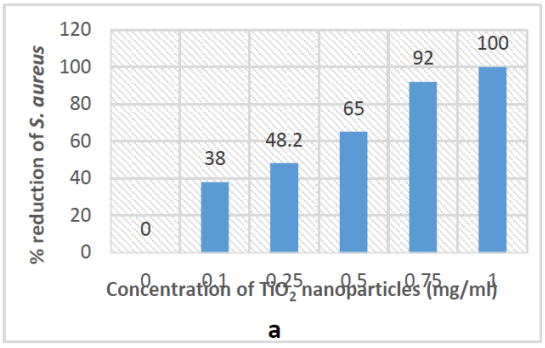


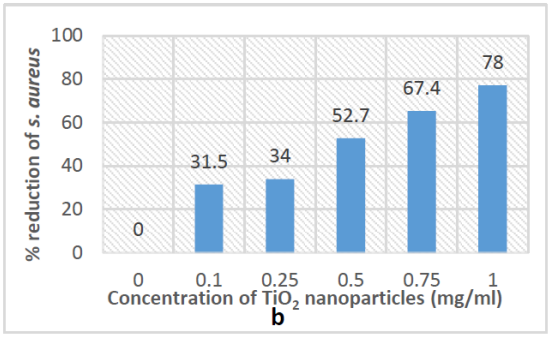
Figure 4: Darw the relation between $(\alpha h\upsilon)_2$ verses photon energy ($h\upsilon$) of TiO_2 NP's calcined at, a) 500˚C, c) 800°C, d) 10000°C

IV. Testing of Bio-activity of TiO_2

The percentage microbial reduction with the TiO_2 nanoparticles against both the species of bacteria Staphylococcus aurous and Pseudomonas aeruginosa is shown in Figs (5) and (6), respectively. Figures 5 and 6 show the relationship between various concentrations of TiO_2 nanoparticles with (Pseudomonas aeruginosa) and (Staphylococcus aurous) bacteria living cells after 24 h of contact. These data show a reduced in number of CFUs with the increase of TiO_2 concentration. TiO_2 activity on these bacteria is therefore likely to be associated with a growth inhibitor effect as a major and simple pathway. This finding highlights the physical impact on cells caused by touch with TiO_2 nanotube, without considering the photocatalytic process. Also, it is consistent with previous references by Leo et al. [11] and Gogniat et al. [12] who observed a finish of bacterial conjugation after closed with TiO_2 NPs without ultraviolet light. Reported by Niederhãusern et al. [13] Self-cleaning of ceramic tiles covered with AG-TiO2 glass also showed antibacterial activity for 24 hours in the dark. According to the standard criterion[14], both anatase and rutile phases of TiO_2 nanoparticles showed excellent antibacterial activity against Staphylococcus aurous with 0.75 mg/ml concentration, and Pseudomonas aeruginosa with 1 mg/ml concentration, which is equal to survival ratio 30%. So, 0.75 mg/ml and 1mg/ml are selected as the optimum concentrations for inactivation of *Staphylococcus(S.) aurous* and *Pseudomonas(P.) aeruginosa* cells, respectively. The results of *P. aeruginosa* Gram-negative bacteria indicated that they are highly resistant to inactivation by TiO_2 nanoparticles and need a higher concentration than *S.aurous* Gram-positive bacteria. The sequence of susceptibility of the bacteria to

inactivation by TiO_2 nanoparticles action is as follows: P. aeruginosa large than S. aureus. This result is in coincident with previous studied order of bacterial susceptibility with Ti: E. coli large than other E.coli . Enterococcus species large than Gram-positive bacteria [15].This configuration may be demonstrated with the fixed body of the bacterial cell wall, in which Gram-positive bacteria has a solid wall, containing several layers of peptidoglycan and teichoic acids. This wall usually is a comparatively thin, but it possesses an additional outer membrane containing lipopolysaccharides and lipoproteins bilayers.The processing of penetration nanoparticles and effect it on bacterial cell through the inhibition of protein or polysaccharides (Muiopeptides) synthesis. However, 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. 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 that become weaker region which suspected that dividing cells. However, Desai and Kowshik [16] reported reverse the order to remove the activation of bacterial .It is compatible with the complex structure of the outer membrane of Gram-negative bacteria, which may protect them against disinfectants [17].





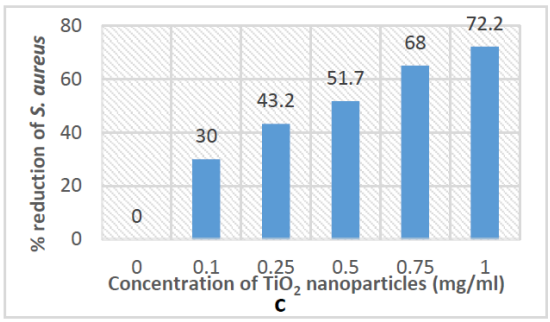
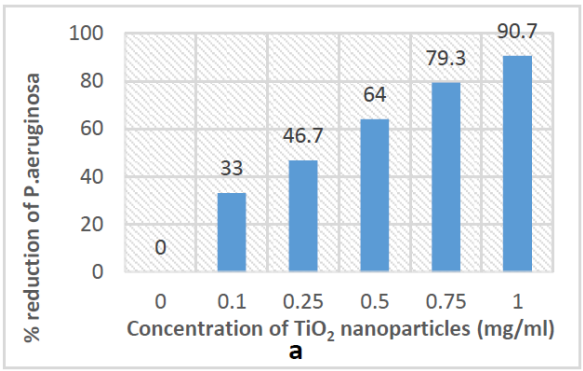
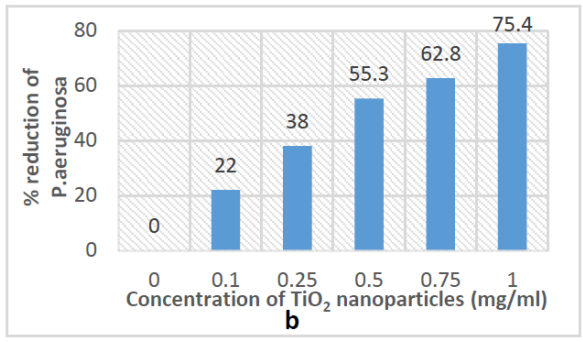


Figure 5: reduction percent of Staphylococcus aurous against different concentrations of TiO_2 nanoparticles (mg/mL of culture) calcined at a) 500 °C, b) 800 °C, and c) 1000 °C





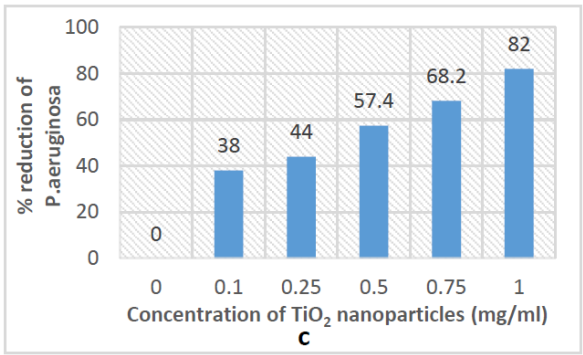


Figure 6: reduction percent of Pseudomonas aeruginosa against different concentrations of TiO_2 nanoparticles (mg/mL of culture) calcined at a) 500 °C, b) 800 °C, and c) 1000 °C

4. Conclusions

Prepared TiO_2 NP's containing hybrid phases (Anatase and Rutile) crystallites were

successfully synthesized by a conventional sol-gel process .We conclude,it was found on the structural and optical properties of TiO_2 NP's depend on the degree of calcination temperature. Increasing the temperature of the calcination, the crystal is improved and the size of crystals becomes greater. Average permeability of all films TiO_2 more than 80% in the wavelength range (400-900) nm and decreases the permeability in the UV region with increasing the degree of calcination temperature.The Eg(optical) depends on the Tc (Inversely proportional).TiO_2 NP's showed excellent antibacterial activity against Staphylococcus aurousand Pseudomonas aeruginosa with inactivation concentration of 0.75 mg/ml and 1mg/ml, respectively.

Acknowledgments

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Adawiay J. Haider has Ph.D. degree in Applied Physics from University of Technology, Baghdad (Iraq) in 2001. She has more than 145 published articles inside and outside Iraq, supervised on 60 Ph.D and M.Sc. Students. She has been a member of Iraqi Society of Physics since 1991, Iraqi Society for Alternative and Renewable Energy Sources and Techniques since 2004, Iraqi Laser Society since 2004 and Iraqi Inventors &inventors Society. Prof.Adawiya has a research group on nanostructures semiconducting thin films produced by PLD, CBD, sol-gel and CVD and their applications and laser photonic group.