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FABRICATION OF PdNPs DECORATED TiO_2 NANOTUBES BY ELECTRODEPOSITION METHOD AS ANTICANCER MATERIALS

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Abstract

In this study palladium nanoparticles were prepared from using five derivatives of Mannich base palladium complexes which have been used as sources of palladium and decorated on TiO_2NTs by using electrodeposition method. Prepared and investigated the physical properties of the anatase TiO_2NTs phase before and after deposition Pd nanoparticles by using X-ray diffraction spectroscopy, transmission electron microscopy (TEM), field emission scanning electron microscope (FE-SEM), energy dispersive X-ray (EDX), and FT-IR technique and comprehensively investigated the effect of cytotoxic depending on the crystal form of TiO_2NTs and Pd\$TiO_2$NTs by using two type of cell lines cancer (MCF-7) and normal (WRL68). The result show that the inhibitory of cancer cell line increases when the palladium nanoparticles are deposited on the TiO_2NTs surfaces.

Keywords: Electrodeposition, Pd nanoparticles, Titanium dioxide nanotube, Cytotoxic assay, Anticancer evolution.

Introduction

Nanoparticles have received much attention in present scenario due to their application in cancer therapy. Based on clinical efficacy, TiO_2NTs has a very wealthy data related to cytotoxicity. The cheapest and most easy method that leads to ordered TiO_2 nanotube array is anodization technique Escada *et al.* (2017). There are many factors that affects in anodizing process such as anodization time Indira *et al.* (2015), temperature Ayal *et al.* (2016). anodization potential Indira *et al.* (2015), and Ayal *et al.* (2016), and the electrolyte Indira *et al.* (2015) and Ayal *et al.* (2016), thus its effect on the diameter, thickness and pores of nanotubes by controlling the anodization conditions, many types of TiO_2 nanotubes such as ripples free or double-wall can be formed Robin *et al.* (2014). In the past, palladium as a member of the platinum group metals was mainly known as an expensive noble metal. Palladium belongs to group ten in the periodic table Saldan *et al.* (2015). Palladium NPs have invaluable mechanical, optical and catalytic, properties that are in widely used in industrial applications as well as anticancer and antibacterial activity Leso and IavicoliInt (2018), Siddiqi, and Husen (2016), and Veena (2018).

Doping of TiO_2NPs with noble metals such as silver, gold or platinum reduces the energy gap and hence the light response in the visible area N., Feng, *et al.* (2013). Generating ROS and their potential applications by doped TiO_2NPs which make them active under visible light have been recently investigated in death of microbial group Jiang *et al.* (2015), and Boxi *et al.* (2016). Thus, some studies have shown that doped TiO_2 can death bacteria without any light illumination and can be activated under normal light Paul *et al.* (2016) and Lin *et al.* (2011).

In the following paper Pd nanoparticles has been used to modify the surface of titania nanotubes and used as an anticancer material from different of Mannich bases palladium complexes at fixed concentration and time. The ligands act as organic material that prevent the accumulation of palladium nanoparticles during the electro deposition. In vitro two types of cancer (breast cancer) and normal cell lines were used.

Material and Methods

All chemicals were purchased from commercial sources $H_2PtCl_6.6H_2O$ (99.9%),S-(1-(benzothiazole-2-ylamino) methyl]-H-benzimidazole-2-yl) 4-nitrobenzothioate (L_1), S-(1-(Pyrazine-2-carboxamido) methyl) -1-H-benzoimi dazole-2-yl) 4- nitrobenzo thiolate (L_2), N-((2-((Morpholinomethyl) thiol)-1H-benzomidazol-yl) methyl) pyrazine-2-carboxamide (L_3), 2-(Morpholin-N-methyl)mercapto-1H-benzimi dazole (L_4), S-(1-Morpholinomethyl)-1-benzoimidazol-2-yl) 4-nitrobenzothio ate (L_5) as the ligands. NH_4F (99.5%), ethylene glycol 99.8% and Ti, Pt foil (99.6, 99.99%) with thickness 0.25 mm. Solvents and reagents were used as received. The nanostructures were characterized by FE-SEM, TEM, XRD, EDX and FT-IR. Transmission Electron microcopy (TEM) was recorded on Philips CM (10). Atomic weight and atomic number of all prepared nanoparticles were carried out by energy dispersive X-ray spectroscopy (EDS) XFlah6-10 Detector –Bruker. X-ray diffraction was measured using Shimadzu ray 6000. The field emission scanning electron microscope measurements were obtained using Hitachi FE-SEM model S-4160, Japan, 0.5 - 20 KV and the FT-IR spectra were recorded using IR Prestige-21, Single Beam Path Laser, Shimadzu (8400).

Preparation of TiO_2NTs and Pd/TiO_2NTs photoelectrodes

TiO_2NT electrodes were produced by anodization according to procedure. For this, titanium sheets (99.9%, Sigma Aldrich) were previously cut (1.0cm × 2cm), degreased by sonication in detergent, deionized (DI) water, ethanol and acetone respectively for 10 min. and dried in an oven at 100 °C for 15 minutes. The TiO_2NTs electrodes were prepared by anodization using two-electrode cell configuration, with Ti foils as anode and Pt foil as a cathode. The anodazing solution containing 0.5 wt.% NH_4F, (99.5%) in anhydrous ethylene glycol (99.8%) and 5.0 Vol.% H_2O at room temperature. The anodized substrate was then soaked in a water bath at 40 °C for 20 minutes to remove the organic electrolyte. The anodization was performed for one hours at 40 V. After the occurrence of the anode then, electrodes were fired at 550 °C during 2hrs in a muffle furnace to transform amorphous TiO_2 into crystalline anatase phase.

The Pd/TiO_2NTs photoelectrodes were prepared by Pd nanoparticle deposition on TiO_2NTs surface by using a conventional two-electrode electrochemical cell with graphite rod and TiO_2NTs template was used for deposition .The electrolyte solution was prepared by dissolving the 2mM from five complexes PdL_1, PdL_2, PdL_3, PdL_4 and PdL_5 in 100 ml mixture solvent (dimethyl formamide, ethanol, deionized water (1:1:1)). Electrodeposition time was set at 3 min., while the PtL_4 at 6min. The electrodeposition voltage was fixed at 7 V and pH=5.5. The prepared Pd \$TiO_2$NTs was washed several times with deionized water to remove the residue of the solutions and then dried in air.

Preparation of complexes

These compounds were prepared according to Alias *et al.* (2017) and (2018).

Cytotoxic assays

Cytotoxicity effect of TiO_2NTs and PdNPs when deposition on TiO_2NTs on MCF-7 and WRL68 cancer cell line, and normal cell lines were done in a sterile area using the biosafety conditions of the airflow cabinet. MCF-7, WRL68 cell lines used in this study were equipped from Biotechnology Center/Al-Nahrain University. The cells were cultured in (MEM) modified eagle's medium with serum ((100 U\ml) of antibiotic, ((100 µg)) of streptomycin/ml in incubator with (5% CO_2 at 37 °C). The survival or death of cells were determined using (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl Tetrazolium bromide ((MTT)) which is diagnosed by using spectro photometer. Plated with 96-well have been sterilized. After twenty-four hours, cells were treated with different concentrations of prepared compounds starting from the lowest concentration and incubated in (5% CO_2) atmosphere with high humidity. After forty-eight hours of compounds exposure, the cells were incubated with (0.5 mg/ml, MTT) distilled water for another four hours at thirty-seven degrees.10% of salt (sodium dodecyl sulphate) then incubated for two hours. Absorption was measured at the wave length 620 nm on a multi-well ELISA plate reader Freshney (2015).

Results and Discussions

The field emission scanning electron microscope (FE-SEM) analysis was used to examine the surfaces and cross section morphology of TNTs template and doped TiO_2 with Pd nanoparticles. In FE-SEM image it is obvious that the formation of a self-organized and uniform nanotube layers with different tube diameters are possible to be achieved. Palladium nanoparticles were distributed uniformly on the surface and some were deposited into the nanotubes using electrochemical deposition method, with diameters in the range from 21 to 32 nm, where palladium nanoparticles gathered on the TiO_2 nanotubes. The palladium nanoparticles have a distorted spherical shape, some of properties and particle size can be noticed in Table (1).

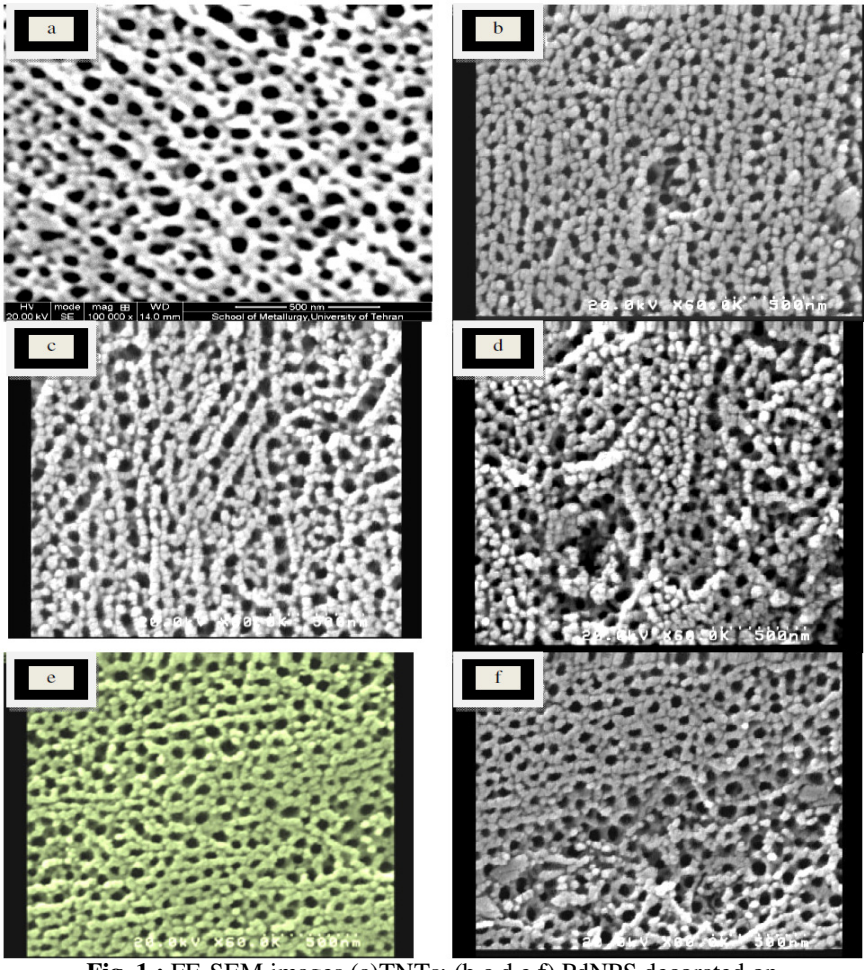

Fig. 1 : FE-SEM images (a)TNTs; (b,c,d,e,f) PdNPS decorated on TiO_2 NTs for all compounds

Table 1 : The range of particle size, outer and inner diameter of PdNPs/TiO_2NTs

Sample	Diameter nm	Inner diameter	Particle size\nm
TiO_2nanotube template	83	68	-----
PdL_1NPs \TiO_2 NTs	89	49	21-30
PdL_2NPs\ TiO_2 NTs	90	48	21-31
PdL_3NPs\ TiO_2 NTs	93	43	24-32
PdL_4NPs \TiO_2 NTs	90	46	22-31
PdL_5NPs\ TiO_2 NTs	91	46	23-30

The following forms show the cross section of TiO_2NTs and heavy metal Pd nanoparticles decorated on it, where the cross section shows the average length of the tubes is about 4 µm as shown in Figures (2). The cross-section shows that the nanotubes are homogeneous and well-covered with small spaces between them, providing a suitable surface for deposition and a large area for penetrating the electrolyte solution.

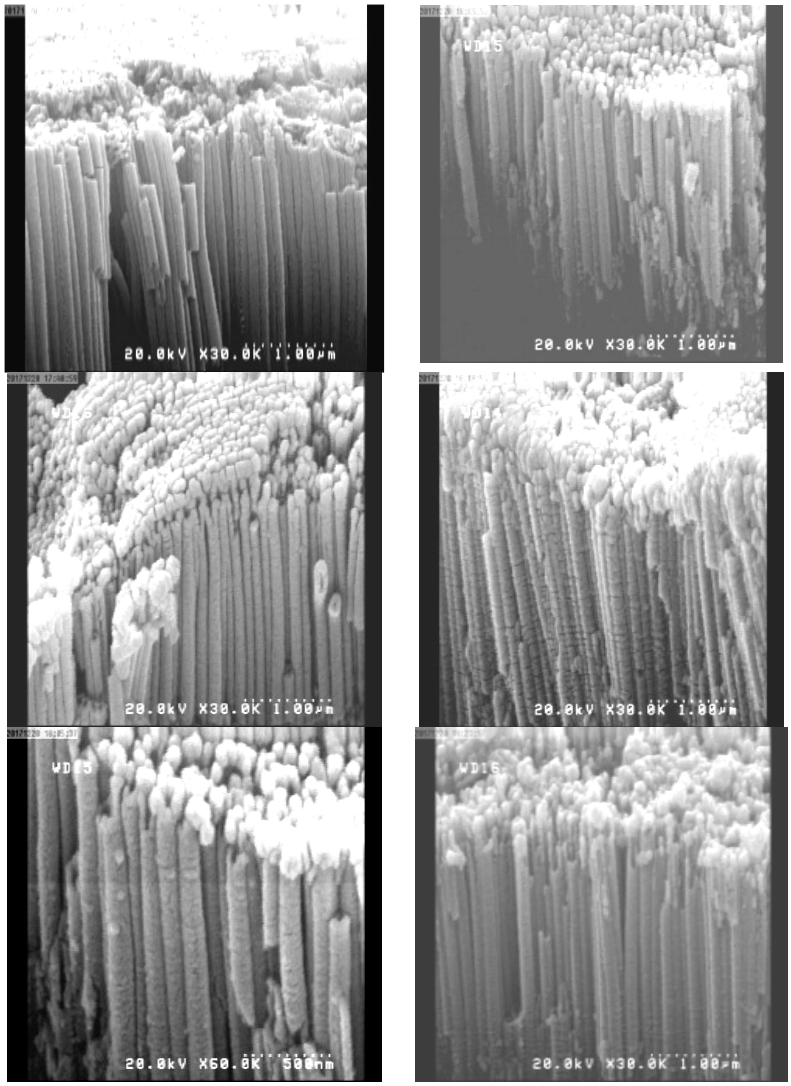

Fig. 2: Cross section images of (a) TNTs; (b) PdNPS deposited into TiO_2 NTs for all compounds

Figure (3 (a) & (b)) shows the TEM morphologies of titanium nanotubes before and after Pd deposition. TiO_2NTs consist of a smooth wall with the average diameters of about 83 nm, which is in agreement with FE-SEM results, after palladium decorated, it can be seen that many Pd nanoparticles have been successfully decorated on the internal and external walls of titanium nanotubes which is in agreement with the cross section FE-SEM results. TEM images of the TNTs and Pd and are represented in Figures (3 (a) & (b)). This result is coinciding with FE-SEM surface results.

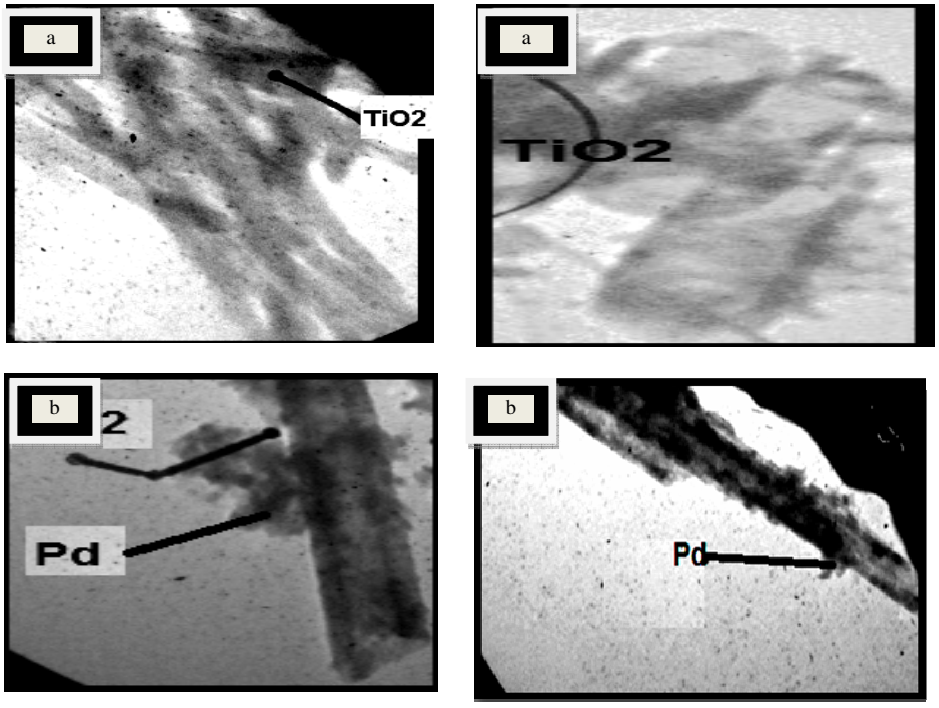

Fig. 3 : TEM images of (a) TiO_2NTs; (b) Pd deposited into TiO_2 NTs

In EDX spectrum, the result is appeared in Figures (4). From the spectrum the presence of four peaks at 4.56, 0.433, 0.421 and 0.325 keV, sequentially are detect. The intense peaks are assigned to the Ti and the less intense refer to oxygen and palladium deposition on it. This result confirms the existence of Pd atoms in the TiO_2 nanotubes. These values were ascertained after comparing them with references [18].

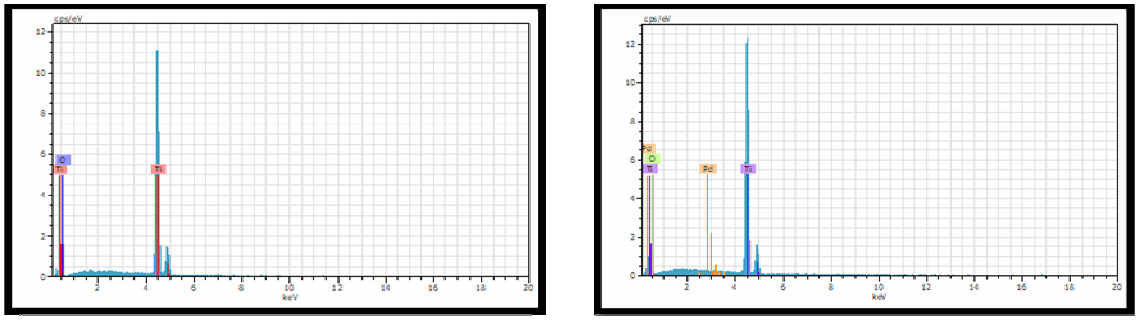

Fig. 4 : EDX analysis of TiO_2NTs and PdNPs/TiO2 nanotubes at 2mM

Raman measurements will prove to be invaluable for evaluating the crystalline phase uniformity of TiO_2NTs .Titanium dioxide nanotubes have distortion octahedral shape in the anatase phase. The primitive unit cell has two TiO_2 units with Ti atoms ((0, 0, 0)) and ((0, 1/2, 1/4)) and O atoms at ((0, 0, u)), ((0, 0, ū)), ((0, 1/2, u+1/4)) and ((0, 1/2, 1/2-u)) giving six active Raman modes: A_1g+2B_1g+3Eg. Anatase phase of TiO_2 has vibrational modes at of Raman transitions and symmetry at 144, 197, 397, 517, 513, 639 cm^{-1}, Figure (5) without any band for other phase (rutile or brookite) [19,20].

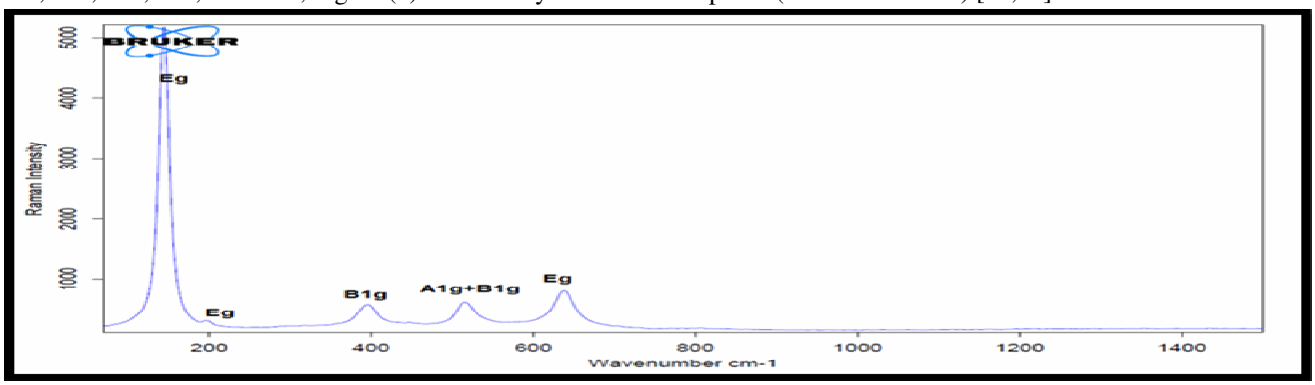

Fig. 5: Raman Spectrum of TiO_2NTs

The structure and phase of the titanium dioxide nanotubes and the metallic salts deposited on it were recorded by X-ray diffraction (XRD) analysis**.** The XRD pattern exhibited the presence of titanium (JCPDS No. 44-1294), anatase (JCPDS No. 21-1272), diffraction peaks of TiO_2 2θ =(25.44), (38.20), (48.29), (54.22), (55.30), (62.82), (70.48) and (75.58)^o Which belong to the (101), (004), (200), (105), (211), (204), (220) and (215) sequentially[14]. Crystallite size of TiO_2 was calculated from Scherer's equation which is equal to 59.6 nm. When deposition of Pd metals does not change in the form of the peaks of the anatase phase which may be attrabuited to low concentration of palladium in the solution of the complexes. These results correspond to the number of researches [21]. A comparison of XRD patterns /Pd samples was shown in Figures (6).

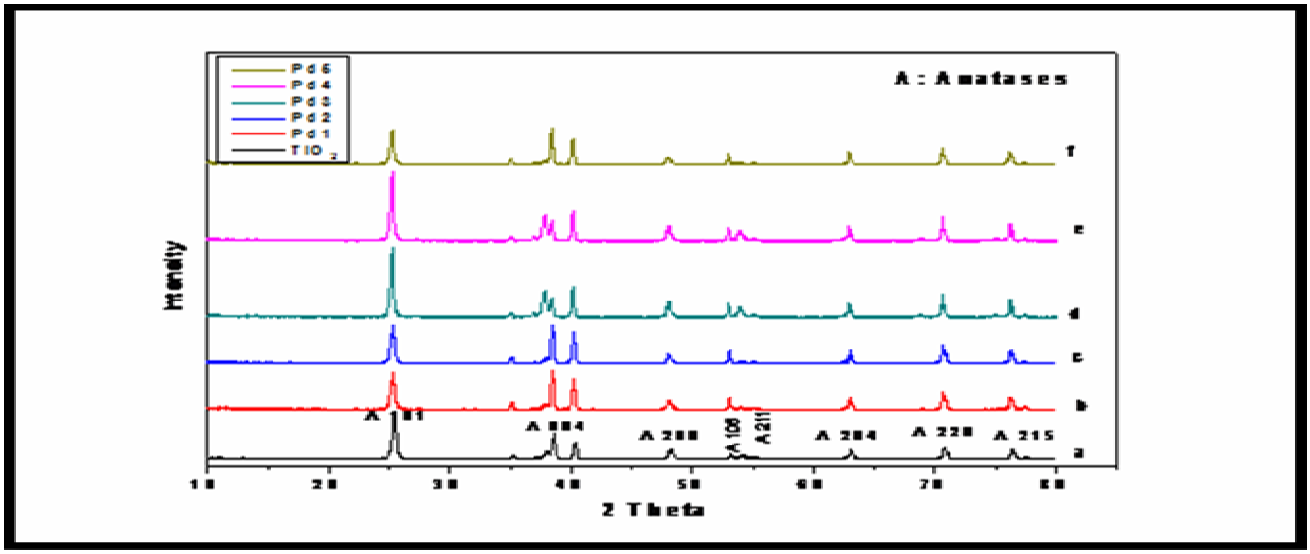

Fig. 6 : X-ray diffraction graphs of TiO_2NTs and Pd nanoparticles when decorated on it (b,c,d,e,f)

From the result of the FT-IR spectrum of titanium dioxide nanotubes showed a broad band in the position 553-435 cm^{-1} which due to the bending bands (**δ**) of (titanium-oxygen-titanium) [20]. The broad band observed around 3477-3338, and1647, 1620 cm^{-1} cm^{-1}, this confirms the existence ν(OH) and **δ**(OH) group present in water molecules [22], which indicate absorption of some of the water molecules on the surface of TiO_2NTs. All Pd nanoparticles in five complexes were showed approximately in the same position of TiO_2 nanotubes, this indicted no precipitate any organic compounds (Mannich bases) on the template of TiO_2NTs.

Interpretation of cytotoxic assay results

The results were done on MCF-7, cell lines shown in Table (2) recorded the cytotoxicity effect of titana and Pd\$TiO_2$NTs for 48 hrs exposure time .There was a significant difference (P<0.05), (t-test=0.00) at all concentrations and compared to normal cell line at concentration (800, 400, 200, 100, 50, 25 and 12.5 µg/ml). Table (2) show the statistical results, and the value of IC_{50} for MCF-7 cancer cell lines and WRL68 normal cell lines. The concentration of Pd \$TiO_2$NT that was required for 50% inhibition of MCF-7 and WRL68 cell inhibition was calculated. All data were expressed as means±standard deviations (SD). The statistical analysis was performed using Independent Samples Test (2-tailed(t-test)) at confidence levels of 95%.

From IC_{50} values, listed in Table (2) it was found that the surface of titanium dioxide nanotubes modified by palladium nanoparticles has a higher toxicity than titanium nanotubes alone.

The toxicity of these nanomaterials Pd\$TiO_2$NTs on cancer cells was observed to be twice as toxic when compared to normal cells.

Table 2 : Statistical data and IC50 Values of Pd\$TiO_2$NTs and TiO_2NTs on cancer (MCF-7) cell lines and normal (WRL68) cell lines in time of exposure 48 hrs

Inhibition rate%(means ±standard deviation± SD				
Conc. µg\ml	**Pd\$TiO_2$NT MCF-7**	**Pd\$TiO_2$NT WRL68**	**TiO_2NT MCF-7**	TiO_2NT WRL**68**
800	61.07±0.848512	29.50±0.560268	49.20±0.136163	34.40±.0577697
400	52.72±0.377097	8.45±0.625290	43.38±.0122317	12.89±0.867106
200	42.38±0.350487	7.65±1.02573	30.97±0.884123	8.84±0.511783
100	34.89±0.308837	6.34±0.729618	20.94±0.051394	7.30±0.237557
50	20.44±0.285001	6.15±0.606028	15.80±0.705143	5.98±0.256689
25	10.87±0.202429	5.90±0.154448	10.50±.0.45254	4.30±0.436534
12.5	9.99±0.387874	3.23±0.116762	7.03±.0417440	3.00±0.325140
IC_{50}	**183**	**403**	**212**	406

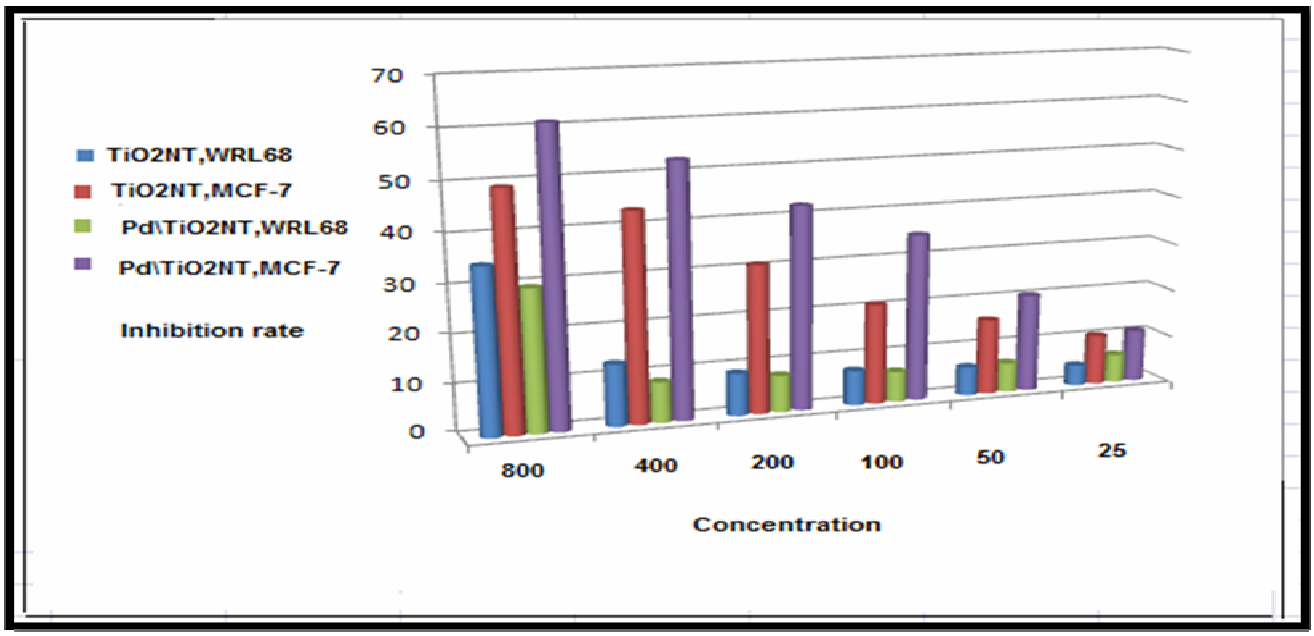

Fig. 7 : The percentage inhibition rate in MCF-7 cell line after treatment withTiO_2NT, Pd\$TiO_2$NT, 48 hrs compared to normal WRL68 cell lines

Conclusion

Pd NPs were electrochemical deposited on titanium dioxide nanotubes support using palladium complexes as a source of palladium and prevent the nanoparticles aggregation. TEM analysis showed distorted spherical morphologies and tubes structures for the Pd nanoparticles and TiO_2NTs respectively. Their elemental composition and chemical states were confirmed using EDX .The Pd\$TiO_2$NTs shows a uniform and well dispersion of Pd NPs through the TiO_2NTs with particle size less than 35 nm. The Pd/TiONTs has higher cytotoxic activity compared with TiO_2NTs when using MCF-7 cancer cell lines.

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Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interest

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