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$_2$NTs by using electrodeposition method. Prepared and investigated the physical properties of the anatase TiO$_2$NTs 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 cytotoxicity depending on the crystal form of TiO$_2$NTs 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$_2$NTs 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$_2$NTs 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 Iavicoli Int (2018), Siddiqi, and Husen (2016), and Veena (2018).

Doping of TiO$_2$NPs 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$_2$NPs 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 deal 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$_2$PtCl$_6$6H$_2$O (99.9%), S-(1-(benzothiazole-2-ylamino) methyl]-H-benzimidazole-2-yl) 4-nitrobenzothioate (L$_1$), S-[(1-(Pyrazine-2-carboxamido) methyl) -1-H-benzoimi dazole-2-yl) 4- nitrobenzo thiolate (L$_2$), N-((2-((Morpholinomethyl) thiol)-1H-benzimidazol-yl) methyl) pyrazine-2-carboxamide (L$_3$), 2-(Morpholin-N-methyl)mercapto-1H-benzimi dazole (L$_4$), S-((1-Morpholinomethyl)-1-benzimidazol-2-yl) 4-nitrobenzothioate (L$_5$) as the ligands. NH$_4$F (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 microscopy (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$_2$NTs and Pd/TiO$_2$NTs photoelectrodes
TiO$_2$NT 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 ºC for 15 minutes. The TiO$_2$NT electrodes were prepared by anodization using two-electrode cell configuration, with Ti foils as anode and Pt foil as a cathode. The anodizing solution containing 0.5 wt.% NH$_4$F, (99.5%) in anhydrous ethylene glycol (99.8%) and 5.0 Vol.% H$_2$O at room temperature. The anodized substrate was then soaked in a water bath at 40 ºC 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 ºC during 2hrs in a muffle furnace to transform amorphous TiO$_2$ into crystalline anatase phase.
The Pd/TiO₂NTs photoelectrodes were prepared by Pd nanoparticle deposition on TiO₂NTs surface by using a conventional two-electrode electrochemical cell with graphite rod and TiO₂NTs template was used for deposition. The electrolyte solution was prepared by dissolving the 2 mM from five complexes PdL₁, PdL₂, PdL₃, PdL₄ and PdL₅ in 100 ml mixture solvent (dimethyl formamide, ethanol, deionized water (1:1:1)). Electrodeposition time was set at 3 min., while the PtL₄ at 6 min. The electrodeposition voltage was fixed at 7 V and pH=5.5. The prepared Pd/TiO₂NTs 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 TiO₂NTs and PdNPs when deposition on TiO₂NTs 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% CO₂ 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% CO₂) 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 TiO₂ 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 TiO₂ 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 TiO₂ NTs for all compounds](image-url)
Table 1: The range of particle size, outer and inner diameter of PdNPs/TiO$_2$NTs

<table>
<thead>
<tr>
<th>Sample</th>
<th>Diameter nm</th>
<th>Inner diameter</th>
<th>Particle size(\text{nm})</th>
</tr>
</thead>
<tbody>
<tr>
<td>TiO$_2$ nanotube template</td>
<td>83</td>
<td>68</td>
<td>---</td>
</tr>
<tr>
<td>PdL$_1$NPs \ TiO$_2$ NTs</td>
<td>89</td>
<td>49</td>
<td>21-30</td>
</tr>
<tr>
<td>PdL$_2$NPs \ TiO$_2$ NTs</td>
<td>90</td>
<td>48</td>
<td>21-31</td>
</tr>
<tr>
<td>PdL$_3$NPs \ TiO$_2$ NTs</td>
<td>93</td>
<td>43</td>
<td>24-32</td>
</tr>
<tr>
<td>PdL$_4$NPs \ TiO$_2$ NTs</td>
<td>90</td>
<td>46</td>
<td>22-31</td>
</tr>
<tr>
<td>PdL$_5$NPs \ TiO$_2$ NTs</td>
<td>91</td>
<td>46</td>
<td>23-30</td>
</tr>
</tbody>
</table>

The following forms show the cross section of TiO$_2$NTs 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](image)

Figure (3 (a) & (b)) shows the TEM morphologies of titanium nanotubes before and after Pd deposition. TiO$_2$NTs 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.
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].

Raman measurements will prove to be invaluable for evaluating the crystalline phase uniformity of TiO$_2$NTs. 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, u)), ((0, 1/2, u+1/4)) and ((0, 1/2, 1/2-u)) giving six active Raman modes: A$_1$g+2B$_1$g+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].
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 (ICPDS No. 44-1294), anatase (ICPDS 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)° 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 attributed 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$_2$NTs and Pd nanoparticles when decorated on it (b,c,d,e,f)](image)

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-titaniuim) [20]. The broad band observed around 3477-3338, and 1647, 1620 cm$^{-1}$ cm$^{-1}$, this confirms the existence v(OH) and δ(OH) group present in water molecules [22], which indicate absorption of some of the water molecules on the surface of TiO$_2$NTs. 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$_2$NTs.

**Interpretation of cytotoxic assay results**

The results were done on MCF-7, cell lines shown in Table (2) recovered 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 mean±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$_2$NTs on cancer (MCF-7) cell lines and normal (WRL68) cell lines in time of exposure 48 hrs

<table>
<thead>
<tr>
<th>Conc. µg/ml</th>
<th>Pd/TiO$_2$NT MCF-7</th>
<th>Pd/TiO$_2$NT WRL68</th>
<th>TiO$_2$NT MCF-7</th>
<th>TiO$_2$NT WRL68</th>
</tr>
</thead>
<tbody>
<tr>
<td>800</td>
<td>61.07±0.848512</td>
<td>29.50±0.560268</td>
<td>49.20±0.136163</td>
<td>34.40±0.0577697</td>
</tr>
<tr>
<td>400</td>
<td>52.72±0.377097</td>
<td>8.45±0.625290</td>
<td>43.38±0.0122317</td>
<td>12.89±0.867106</td>
</tr>
<tr>
<td>200</td>
<td>42.38±0.350487</td>
<td>7.65±1.02573</td>
<td>30.97±0.884123</td>
<td>8.84±0.511783</td>
</tr>
<tr>
<td>100</td>
<td>34.89±0.308837</td>
<td>6.34±0.729618</td>
<td>20.94±0.051394</td>
<td>7.30±0.237557</td>
</tr>
<tr>
<td>50</td>
<td>20.44±0.285001</td>
<td>6.15±0.606028</td>
<td>15.80±0.701543</td>
<td>5.98±0.256689</td>
</tr>
<tr>
<td>25</td>
<td>10.87±0.202429</td>
<td>5.90±0.154448</td>
<td>10.50±0.45254</td>
<td>4.30±0.436534</td>
</tr>
<tr>
<td>12.5</td>
<td>9.99±0.387874</td>
<td>3.23±0.116762</td>
<td>7.03±0.0417440</td>
<td>3.00±0.325140</td>
</tr>
<tr>
<td>IC$_{50}$</td>
<td>183</td>
<td>403</td>
<td>212</td>
<td>406</td>
</tr>
</tbody>
</table>
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$_2$NTs 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$_2$NTs with particle size less than 35 nm. The Pd/TiO$_2$NTs has higher cytotoxic activity compared with TiO$_2$NTs 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

References


