

CONSIDERATION REGARDING THE USE OF TiO₂ DOPED NANOPARTICLES IN MEDICINE

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In present work, nanocrystalline powders of TiO₂ undoped and doped with Pt and Au ions respectively were synthesized. The synthesis was performed under hydrothermal conditions, at a temperature of 493 K for 5h. Obtained nanocrystalline powders were characterized by X-ray powder diffraction (XRD) and transmission electron microscopy (TEM). The citotoxic effect of TiO₂ nanoparticles undoped and doped with Pt and Au ions respectively on human eritroleukemia tumoral cell line (K562) was investigated.

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1. Introduction

The most important industrial compound of Ti is the titanium dioxide, TiO₂ [1]. TiO₂ was so far extensively used as a photocatalyst for the investigation of conversion of the solar energy into chemical energy and in environmental applications, i.e. the photooxidation of organic pollutants in drinking and residual waters [2,3]. Several titanium compounds such as the dioxide, salicylate and tannate, are nowadays used as important components of many pharmaceutical and cosmetic products [1]. The cyclopentadienyltitanium (IV) derivatives (titanocene complexes) have been reported to constitute new type of antitumor agent showing antitumor activities against certain experimental animal tumors [4,5]. Wealth data regarding biochemistry, toxicokinetics, human and animal toxicity, levels of tolerance, evaluation of healing risks to humans have already been gathered and a number of reviews are available [1]. However, there are rather few reports upon the applications of TiO₂ in biological and particularly medical studies. In an early study on the applications of TiO₂ in biology Matsunaga et al. [6] showed that microbial cells in water could be efficiently killed when reaching in contact with a platinum-loaded titanium oxide powders (TiO₂/Pt) and irradiated with a near-UV light for 60 to 120 min. Cai et al. [7] showed for the first time that the in vitro cultured HeLa cells could be effectively killed in the presence of TiO₂ with UV irradiation using a 500 watt mercury lamp.

The hydrothermal method with an aqueous solvent as a reaction medium is environmentally friendly because the reactions are carried out in a closed system [8,9]. It is important that hydrothermally obtained powders could be produced with a different microstructure, morphology and phase composition by varying parameters such as temperature, pressure, duration of process, concentration of chemical species, concentration of a solution and pH of solutions [8-10].

In recent years, in contrast to many studies on the use of TiO₂ powder for photodecomposition of organic pollutants [11-18], only few studies have investigated the

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application of TiO₂ in life science, especially in the field of cancer treatment [19-21]. Still, the spread of cancer disease is alarming i.e. the incidence of colon cancer is rising in China. Despite the fact that surgery is used currently, medical doctors have recognized and admitted its limitations. The basic way to treat cancer usually includes radiation therapy and chemical therapy, which may, however, give rise to severe side effects in human body. Therefore, tremendous efforts are concentrated on different directions for finding efficient means for cancer treatment.

In this study an attempt to investigate the use of TiO₂ as new therapeutic agent for cancer is presented. Ls-174-t cells were used as experimental substrates. The photocatalytic killing effect of TiO₂ nanoparticles on malignant cells and its killing mechanism were investigated.

Cultured human colon carcinoma cells were effectively killed when exposed in vitro to photoexcited TiO₂ nanoparticles. The concentration of TiO₂ essentially affected the photocatalytic killing effect. The photocatalytic killing effect of TiO₂ nanoparticles on human colon carcinoma cells documented in this study strongly suggests that treatment of cancer based on the use of TiO₂ nanoparticles and light irradiation might be effective. In the light of these findings, it can be envisaged that an anticancer treatment modality based on local or regional exposure of the tumor to TiO₂ nanoparticles, followed by light irradiation of the tumor region could be considered [22].

In this work, we will test citotoxic effect of TiO₂ nanoparticles undoped and doped with Au and Pt ions synthesized by hydrothermal method.

2. Samples preparation and experimental methods

In this experiment were prepared 3 samples: one of TiO₂ undoped and two of TiO₂ doped with Au and Pt ions.

TiO₂ was obtained by hydrothermal procedure using 5 ml TiCl₄ (has obtained from Merck, 99%) 50 ml, oxalic acid and 1%wt dopant solution. TiCl₄ was added drop by drop onto oxalic acid, under continuous mixing conditions, at room temperature over the entire experiment. After 10 minutes of mixing the dopant solution (1%wt PtCl₃/1%wt AuCl₄H) was added.

The obtained solution has been placed inside a teflonated autoclave and the teflonated autoclave was placed in a metallic autoclave; the process evolved at 493 K, 5h. The subsequent preparation of the powder consists in filtration, washing with distilled water and drying at 50^oC.

In order to study the effect of TiO₂ exposure on tumoral cells the experiments were performed on human eritroleukemia tumoral cell line (K562). The cells were cultured on plates with basal culture media (RPMI 1640) supplemented with 10% fetal calf serum (FCS), antibiotics and antimicrobics and incubated at 37°C, in 5% CO₂ atmosphere.

K562 cells were loaded on 24 well plates (80000 cells/well), and after a 3 hours incubation test, the 20 µL from each TiO₂ compounds suspension were added to the wells, according to the Table 1.

For each TiO₂ compounds, the 0.005 g of powder were suspended in 15 ml distilled water and followed by 20 minutes ultrasonication. All the suspensions were sterile filtered (through 0.2 µm filters) before being added to the plate.

After 24 incubation period (37°C, 5% CO₂) the medium was completely changed, cells were suspended in media w/phenol red (RPMI 1640+10%FCS), without addition of TiO₂ compounds.

The cells were directly exposed to UV light for 30 minutes and the plates were incubated for another 24 hours (37°C, 5% CO₂) prior to assess the cell survival rate by MTT test. The control plate, unexposed to the UV light was prepared also.

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), is taken up into cells and reduced by a mitochondrial dehydrogenase enzyme to yield a purple formazan product which is largely impermeable to cell membranes, thus resulting in its accumulation within healthy cells. Solubilization of the cells results in the liberation of the product which can readily be detected using a simple colorimetric assay.

200 µL of cell culture were taken from each well and loaded on 96 well plates. 15 µL of MTT reagent/well were added and 150 µL/well of lysis agent, after a 3 hours incubation. Data

acquisition was carried out on an ELISA (BIORAD) plate reader running at 570 nm test wavelength and 690 nm reference wavelength.

3. Results and discussion

In order to test the characteristics of the obtained TiO₂-based nanocrystals and eventually their amenability as reliable nanomaterials for further biological studies, X-ray diffraction (XRD) and transmission electron microscopy (TEM) analyses have been performed.

XRD patterns of TiO₂ undoped and doped with Au and Pt ions prepared by hydrothermal method at 493K, 5h are displayed in Fig. 1 and 2. X-ray diffraction was performed with BRUKER D8 ADVANCE diffractometer with monochromator using copper tube. The analysis was run at room temperature, at an operating voltage of 40kV, and a current intensity of 30 mA.

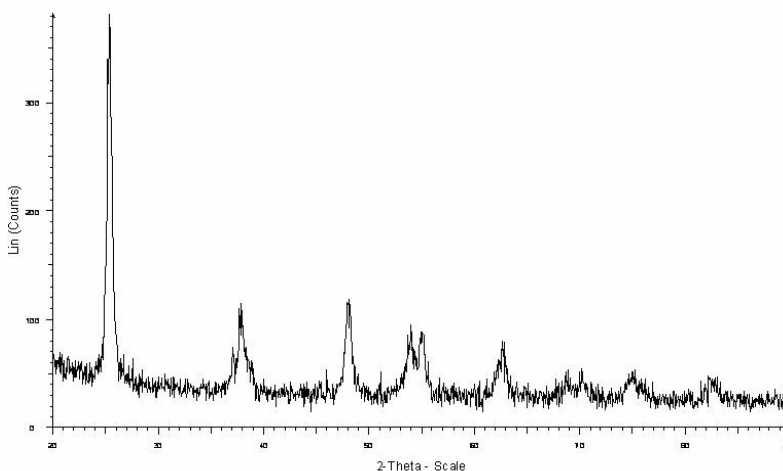


Fig. 1. X-ray diffractogram of TiO₂ undoped, prepared by hydrothermal method ($T=493K$, $t=5h$)

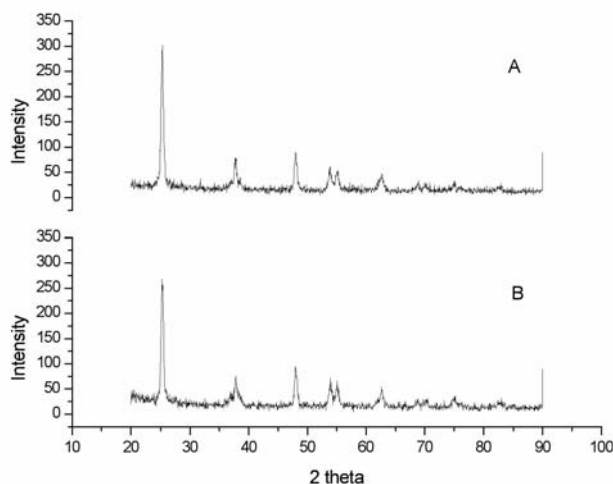


Fig. 2. X-ray diffractogram of TiO₂ doped: A). with Au ions, B). with Pt ions, prepared by hydrothermal method ($T=493K$, $t=5h$).

Diffraction patterns indicate that in case of TiO₂ undoped and doped with 1% Au ions, and 1% Pt ions respectively, the hydrothermal treatment yield a metastable nanocrystalline anatase.

In Fig. 3 the TEM images depicting the particle distribution of the nanocrystalline TiO₂ particles undoped and doped with Au and Pt ions, prepared by hydrothermal method are presented.

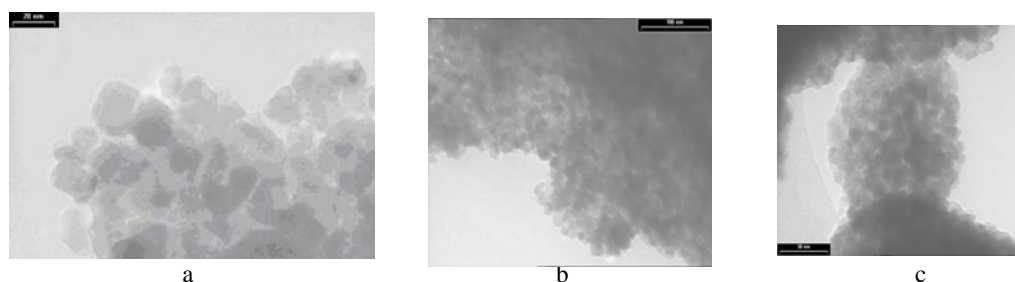


Fig. 3. TEM images of nanocrystalline TiO₂ particles undoped (a) and doped with (b). Au ions; (c). Pt ions, prepared by hydrothermal method ($T=493K$, $t=5h$).

TEM images clearly reveal that the crystals have the specific form of anatase structure. A relatively homogeneous distribution of the particles can be deduced from TEM images, while the dimensional dispersion is relatively small, 12 nm size nanocrystals being the upper limit.

Table 1.

Compounds	Amount (μL)	UV exposed absorbance (570/690 nm)	Unexposed samples absorbance (570/690 nm)
CELLS ONLY	NA	0,096	0,113
R	NA	0,12	0,115
TiO ₂ -Au	20 μL	0,169	0,131
R	20 μL	0,147	0,142
TiO ₂ -Pt	20 μL	0,125	0,121
R	20 μL	0,142	0,115
CELLS ONLY	NA	0,121	0,125
R	NA	0,113	0,111
TiO ₂	20 μL	0,125	0,126
R	20 μL	0,113	0,115
TiO ₂ -Au	20 μL	0,125	0,126
R	20 μL	0,135	0,121
TiO ₂ -Pt	20 μL	0,131	0,131
R	20 μL	0,126	0,125
CELLS ONLY	NA	0,131	0,115
R	NA	0,115	0,121
TiO ₂	20 μL	0,13	0,131
R	20 μL	0,109	0,111
TiO ₂ -Au	20 μL	0,108	0,096
R	20 μL	0,131	0,125
TiO ₂ -Pt	20 μL	0,135	0,125
R	20 μL	0,129	0,123
CELLS ONLY	NA	0,131	0,142
R	NA	0,125	0,115

R- replicates; NA – not added

The study of the cytotoxic effect was further carried out under the procedure described above (see Experimental section). To assess the best suited TiO_2 nanocrystal types for cancer cell killing a comparative investigation of the cell survival in TiO_2 , Au doped and Pt doped TiO_2 environment was considered. The obtained data are summarized in Table 1. As documented in Table 1 a considerable decrease of the survival rate of cells treated with metal-doped TiO_2 particles and exposed to the UV light could be obtained. However, no significant differences in survival rate between cells treated with Au-doped TiO_2 powder and the cells incubated with Pt-doped TiO_2 particles was noticed (Fig. 4). On the other hand, changes of cell survival rate in wells with cells incubated with TiO_2 particles, comparatively to cells only could not be observed. For the control plate, unexposed to the UV light, this cytotoxic effect could not be identified even in the wells treated with Au and Pt doped TiO_2 compounds. (Table 1). These set of data strongly suggests that doping of the TiO_2 with Au and Pt essentially contributes to the killing of the cancer cells as compared to un-doped TiO_2 . Though, basically the survival rate did not drastically decreased under the used environmental conditions it can be envisaged that by carefully altering the conditions used for cell and TiO_2 preparation a substantial improvement of the killing effect could be obtained.

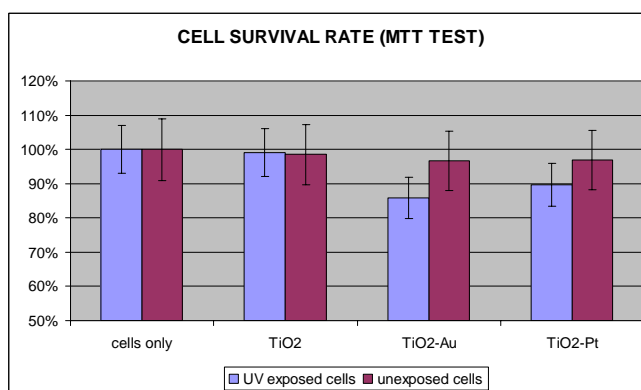


Fig. 4. The results of MMT test (K562 cells, incubated with various TiO_2 doped particles).

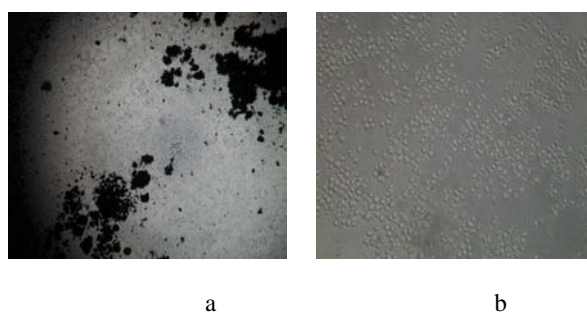


Fig. 5. K562 cells incubated with TiO_2 -Au particles (a) and with TiO_2 -Au suspension (b), direct microscopy, ob. 20x.

In this experimental model the TiO_2 insoluble particles were suspended according to an efficient protocol. The characteristic particle aggregates which could be identified at the microscopic examination in our previous experiments were absent in this case (Fig. 5) and the shield effect of the aggregates was completely removed. The UV exposure was performed after suspension removal from cell media. In this concern we suggest that the cytotoxic effect was induced by the particles which entered into the cells in the first 24 hours interval.

4. Conclusions

TiO₂ nanocrystals undoped and doped with Au and Pt ions were synthesized successfully by hydrothermal method. Results of structural analysis shows that TiO₂ nanocrystals undoped and doped with Au and Pt ions has the specific form of pure anatase structure and the dimension about 12 nm. Results obtained in study of citotoxic effects of TiO₂ nanocrystals strongly suggests that doping of the TiO₂ with Au and Pt essentially contributes to the killing of the cancer cells as compared to un-doped TiO₂.

Thus our results represent a promising basis for the future developments in the field of applications of TiO₂ based nanomaterials for cancer treatment.

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