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Titanium Dioxide Modified with Glutathione as Potential Drug Carrier with Reduced Toxic Properties

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***Abstract*—**The paper presents a process to obtain glutathione-modified titanium oxide nanoparticles. The processes were carried out in a microwave radiation field. The influence of the molar ratio of glutathione to titanium oxide and the effect of the fold of NaOH vs. stoichiometric amount on the size of the formed TiO_2 nanoparticles was determined. The physicochemical properties of the obtained products were evaluated using dynamic light scattering (DLS), transmission electron microscope- energy-dispersive X-ray spectroscopy (TEM-EDS), low-temperature nitrogen adsorption method (BET), X-Ray Diffraction (XRD) and Fourier-transform infrared spectroscopy (FTIR) microscopy methods. The size of TiO_2 nanoparticles was characterized from 30 nm to 336 nm. The release of titanium ions from the prepared products was evaluated. These studies were carried out using different media in which the powders were incubated for a specific time. These were: water, SBF and Ringer's solution. The release of titanium ions from modified products is weaker compared to unmodified titanium oxide nanoparticles. The reduced release of titanium ions may allow the use of such modified materials as substances in drug delivery systems.

***Keywords*—**Titanium dioxide, nanoparticles, drug carrier, glutathione.

I. Introduction

MOST of the currently used drugs belong to low molecular weight compounds, which are quickly metabolized and excreted from the body. Attempts are made to solve these problems by searching for new preparations with more selective activity or by using drug carriers. Although the role of carriers in the delivery of active substances is underestimated, there is a growing conviction that the implementation of the concept will bring significant progress in the treatment of many diseases, including cancer [1].

Drug carriers based on metal nanoparticles and metal oxides are an alternative to currently used inorganic drug carriers [2], [3]. The advantage of their application is a developed proprietary surface and the possibility to modify their surface by creating bonds between the active substance and the carrier, which allows for gradual release of the active substance after entering the organism. Bioconjugation of drugs and carriers increases the time of drug circulation in blood and ensures stable and controlled release at the target site. The use of a carrier protects against rapid removal of the active substance from the cell by the transmembrane drug pump. Nanoparticles are surrounded by the cell membrane and transported to the site of endosomal vesicles, which release chemotherapeutics [4], [5]. On the other hand, it is necessary to identify possible adverse effects of interactions between nanoparticles and organisms.

Non-toxic nanoparticle-based carriers of active substances include zinc oxide (ZnO) and titanium oxide (TiO_2). The choice of parameters and methods of their synthesis makes it possible to obtain particles of controlled size and shape [6]. However, the larger the surface area of nanoparticles, the higher the degree of ion release from their surface. Currently, many methods are known for the preparation of TiO_2 NPs. Among them, the most popular are sol-gel, hydro- and solvothermal methods and electrochemical methods [7].

The object of the present work was to obtain a modified form of titanium(IV) oxide nanoparticles by depositing glutathione on their surface. The modification of the TiO_2 surface aimed at increasing the affinity of titanium oxide as a drug carrier for cancer cells and slowing down the release of the active substance from the resulting complex. The modification was also aimed at reducing the toxicity of titanium oxide itself, which manifests itself in the release of titanium ions [3]. In the present study, tadalafil, which is a model drug, was used and there are several reasons in favor of replacing the target active substance with it. The structure of tadalafil is based on that of indole derivatives, which are one of the anticancer drug groups.

II. Materials And Methods

A. Materials

Titanium(IV) isopropoxide (TIPO, ≥ 97.0%) and sodium hydroxide (≥ 98.0 %) were used to synthesize titanium oxide nanoparticles. The material was stabilized by glutathione (99.0%). Tadalafil (Pharmaceutical Secondary Standard; Certified Reference Material) was used as reference material of active substance.

B. Methods

In the first step basic hydrolysis of titanium isopropoxide in the presence of NaOH was carried out. The process led to the formation of titanium hydroxide. For this purpose, in the presence of ultrasonification (ultrasound homogenizer, Hielscher UP400St, Germany (40 W)), titanium(IV) isopropoxide was dropped to the aqueous solution of sodium hydroxide. After two minutes of homogenization, an aqueous solution of glutathione was added, and the whole solution was homogenized for another 2 min. Next, the powder of tadalafil was introduced to the reaction mixture, and the obtained suspension 1 was mixed with a magnetic stirrer for 5 min. Then,

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the Teflon vessel was placed in the microwave reactor at 180°C, p=15-20 bars for 10 min (Magnum v2, Ertec, Poland). In the presence of microwave energy, titanium hydroxide particles were dehydrated to titanium(IV) oxide and water.

The amounts of all reagents were calculated so the mass ratio of tadalafil to the whole TiO_2-Tad complex was equal to 1.0:3.5. Three modified materials were obtained, differing in the amount of reagents used for their synthesis. Specific values of reagent amounts are provided in Table I.

C. Instrumental Analysis

The phase composition of the forming titanium oxide was confirmed by XRD analysis. The surface topography of the products was examined by elemental analysis (TEM-EDS). In addition, DLS technique was used to evaluate the size and stability of the obtained nanoparticles. The specific surface area and pore volume and size were measured using the BET method.

The stability of titanium nanoparticles was investigated by analyzing the release of titanium ions from the obtained products. Among others, the effect of the presence of glutathione was compared to the unmodified material.

For this purpose, the presence of titanium ions was studied over time, during the mixing of TiO_2 nanoparticles in water (0.5% w/v, 37°C). The concentration of Ti^{4+} ions was determined using atomic absorption spectroscopy (ASA) analysis in this acceptor fluid.

TABLE I
PROCESSES PARAMETERS (T=150°C, T = 5 MIN)

Sample	n GLU: n TiO_2	fold of NaOH vs. stoichiometric amount
A1	0.02	1
A2	0.02	2
A3	0.02	3
A4	0.11	1
A5	0.11	2
A6	0.11	3
A7	0.20	1
A8	0.20	2
A9	0.20	3
A0	0.00	1

III. RESULTS

The XRD diffractograms (Fig. 1) show four reflections which indicate the receipt of titanium (IV) oxide in all samples. Reflections were clear, and no additional reflections were observed, proving the high purity of the obtained products. The average crystallite size of the TiO_2 nanoparticles ranged from 4.1 nm to 6.2 nm. The particle size corresponds to the results obtained from the SEM analysis (Fig. 2). The nanoparticles appear as agglomerates with spherical or irregular particle shape. The materials are characterized by a low degree of crystallinity.

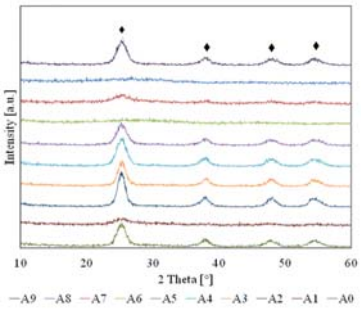

Fig. 1 XRD diffractograms indicating obtaining TiO_2

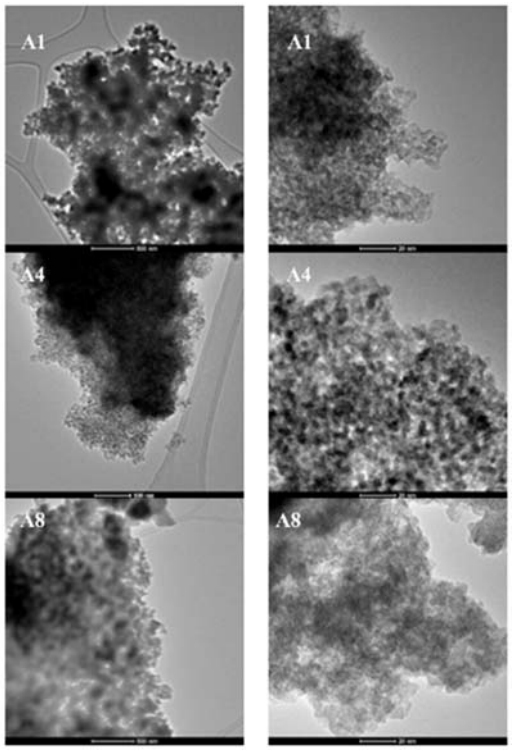

Fig. 2 HR-TEM microphotography of titanium oxide nanoparticles modified with glutathione

Based on the BET (Fig. 3) analysis, it was found out that modified titanium oxide nanoparticles have greater specific area than not modified product (BET surface area for A0 - 228.89 m^2/g). This, both size and volume of pores have been larger (Table II).

TABLE II
PROPERTIES OF TiO_2

	A1	A4	A8
nGLU: nTiO_2	0.02	0.11	0.20
fold of NaOH vs. stoichiometric amount	1	1	2
BET Surface Area [m^2/g]	383.2441	231.9245	263.7653
BJH Adsorption cumulative volume of pores [cm^3/g]	0.638250	0.312864	0.440938
BJH Desorption cumulative volume of pores [cm^3/g]	0.376991	0.228980	0.273754
BJH Adsorption average pore diameter (4V/A) [nm]	1.0957	1.5119	1.2905
BJH Desorption average pore diameter (4V/A) [nm]	3.2858	2.9668	3.6774

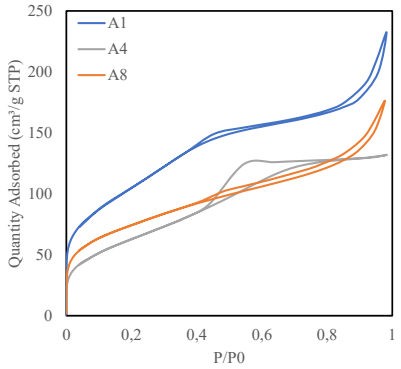

Fig. 3 Isotherms linear plot of TiO_2 nanoparticles

The size of obtained particles measured by DLS technique was different and it was in the range of 29.7–336 nm (Fig. 4). What is more, the values of electrokinetic potential were greater than 20 mV in all cases, which is satisfactory result. The effect of the molar ratio of glutathione to TiO_2 and the effect of excess NaOH on the size of TiO_2 nanoparticles is shown in Fig. 5.

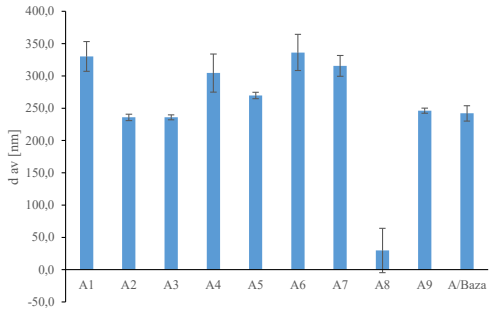

Fig. 4 The size of TiO_2 nanoparticles

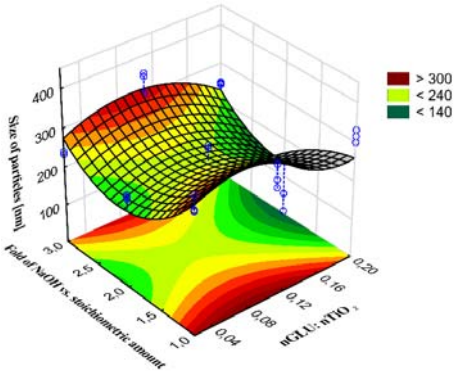

Fig. 5 Effect of molar ratio of glutathione to TiO_2 and effect of excess NaOH on the size of TiO_2 nanoparticles

Fig. 6 shows the FTIR analysis spectra of TiO_2 nanoparticles obtained. All curves have a broad absorption band at 3270 cm^{-1}, which is related to the stretching vibrations of the O-H groups from adsorbed water. There is a characteristic peak around 400 cm^{-1} and this represents the stretching vibrations of the Me-O metal oxides. In the spectra of products glutathione peaks are visible. These occur at 1450 cm^{-1} and can be attributed to C-O-C vibrations and OH and CH vibrations, respectively. The presence of tadalafil in all products is confirmed by peaks occurring at about 1650 cm^{-1}. These represent N-H and C=O vibrations.

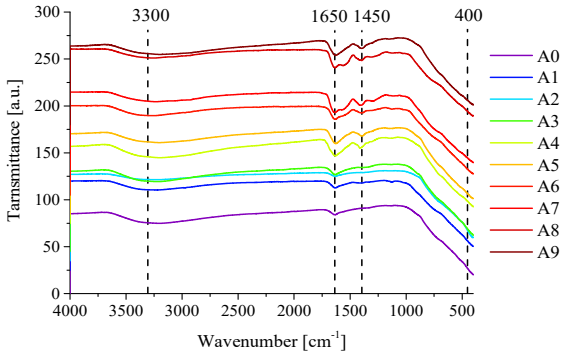

Fig. 6 FTIR spectra of TiO_2 nanoparticles modified by glutathione

TABLE III
MEAN VALUES OF Δ_{Ti4+} AFTER 80 MIN

Sample	$\Delta_{Ti4+\,(mean\ value)}$, %
A1	8,98
A2	-48,84
A3	0,70
A4	25,13
A5	-34,00
A6	9,00
A7	74,89
A8	71,17
A9	-61,02

Based on the analysis of Ti^{4+} ion release from TiO_2 nanoparticles, the modified TiO_2 nanoparticles A1, A3, A4, A6, A7 and A8 were found to be less soluble and showed limited Ti^{4+} release. The difference in titanium release from the modified samples compared to the base sample was calculated for all time intervals. Equation 1 was used for this purpose. The average value of ΔTi^{4+} was then calculated for each product. The results are presented in Table III.

$$\Delta_{Ti^{4+}} = \left(1 - \frac{C_{(Ti^{4+})_{mod.sample}}}{C_{(Ti^{4+})_{base}}}\right) \cdot 100\% \quad (1)$$

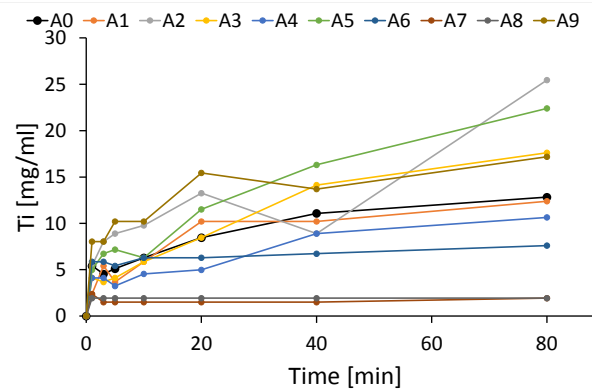

Fig. 7 The concentration of TiO_2 in filtrates. depending on the elution time

The greatest difference between the base sample and the modified sample is observed for products A8 and A7 (Fig. 7). In this case the zinc release was inhibited above 70%. This preparation was obtained using glutathione in a ratio of 0.2:1 and the amount of NaOH relative to the stoichiometric amount was 2 or 3. This confirms the need for a modifier to protect the ions from release.

IV. Conclusion

Analysis of the leachability of Ti^{4+} from glutathione-modified TiO_2 nanoparticles indicates that the addition of a stabilizing substance reduces the release of Ti^{4+} ions over time by more than 70%. After 20 min, the metal ion concentration stabilizes, with the lowest Ti^{4+} concentrations observed for TiO_2 with the addition of 0.2 nGLU/nTiO_2 and or 3 NaOH:nTiO_2. With the best process parameters, the TiO_2 concentration is 3 times lower compared to unmodified TiO_2 nanoparticles.

Acknowledgment

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