

The Ag Influence on the Surface States of TiO₂, Optical Activity and Its Cytotoxicity

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Ag/TiO₂ nanoparticles (NPs) were prepared by a simple chemical deposition method using silver nitrate and suspension of TiO(OH)₂. Silver NPs were deposited on the surface and inside of TiO₂ NPs depended on the Ag concentration. The Ag/TiO₂ composites were characterized by X-ray diffraction, transmission electron microscopy, scanning electron microscopy, and Raman spectroscopy. The cell viability was assessed using an MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay after NPs exposure, since only viable cells have functional mitochondrial dehydrogenase enzymes that can reduce MTT to formazan. The shift of the E_g mode from 143 to 150 cm⁻¹ and FWHM from 12 to 19 cm⁻¹ was stated due to a decrease in TiO₂ crystallites. Optical activity increased after loading with Ag because metal particles offered electron traps to reduce recombination of holes and electrons, especially Ag loading of 8 wt. %. A Schottky barrier is formed in the Ag-TiO₂ contact region, which improves charge separation and thus slows down the recombination of photogenerated electrons and photogenerated holes. The obtained results indicate a lower toxicity of NPs in a glycerin + water suspension, regardless of the introduction of silver molecules in an amount of 4 or 8 wt. %, their CC₅₀ values were 50 and 3.9-58.5 μg/ml for MDBK and MDCK cells, respectively. The obtained Ag/TiO₂ NPs due to their optical activity will be examined as an antifungal material for the inhibition of bacteria and viruses in water.

Keywords: Ag/TiO₂, Irradiation, Raman, Defects, Optical activity, Cytotoxicity, MTT.

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1. INTRODUCTION

Nowadays, the prevalence of viral infections in the world and, in particular, the scale of the COVID 19 pandemic, has led to the rapid development of the anti-virus industry, which, along with the development of vaccines and personal care products, includes new composite materials and devices capable of ensuring the safety of transport and public spaces. Oxide semiconductors have attracted scientific interest for many years because of the number of organic substances that can be oxidized to CO₂ and water at room temperature with oxide catalysts in air, water under UV or visible light illumination [1-9]. Contaminants are largely converted into stable inorganic compounds, such as water, carbon dioxide and salts, i.e., they undergo mineralization. UV light excites electrons from the valence band into the conduction band; the resulting electron/hole pairs can then migrate to the surface and initiate redox reactions with adsorbed organics [6]. As such, TiO₂ photocatalytic activity under Vis (> 400 nm) can be increased by doping; and numerous groups utilize metal ions, such as Fe, Ag, C-S, to hinder the recombination process [7-12]. A redshift of the UV absorption edge was registered for Fe-doped anatase, resulting in a significant decrease in its bandgap from 3.2 to 2.7 eV. The photocatalytic destruction of the organic dye Safranin T over the prepared Fe-doped TiO₂ under UV

irradiation was investigated. The influence of various energetically nonequivalent active sites on the surface of anatase and rutile on their photoactivity was considered [3]. Ag-TiO₂ was deposited on glass substrates with different dopant concentrations (1, 3, 5, 7 and 10 %) and annealed at 550 °C [13]. The crystal structure and phase formation of Ag-TiO₂ was examined using XRD. HRTEM analysis of pure and 5 % Ag-doped TiO₂ thin films revealed that the particles are spherical with sizes around 23.8 and 11.6 nm, respectively. Ag-doped TiO₂ nanoparticles (NPs) exhibit characteristic photoluminescence (PL) corresponding to the visible spectral range with excitation at 325 nm. The intensity of luminescence emission decreases upon doping with silver ions due to a decrease in the bandgap of TiO₂ from 3.2 to 2.7 eV. Morphology-controlled rutile titanium (IV) oxide (TiO₂) and anatase TiO₂ were usually prepared by a hydrothermal method, and their surfaces were selectively loaded with Au, Ag, and Au-Ag bimetallic NPs by photodeposition to obtain visible light-responsive photocatalysts [14]. Ag-doped TiO₂ has been proven to increase the TiO₂ activity in the visible region by the photoreduction method. Radiophotography of wastewater has been reported as a cheap source of Ag (I) that contains [Ag(S₂O₃)₂]³⁻ [6]. The effort to replace expensive AgNO₃ as an Ag precursor, as well as to convert toxic [Ag(S₂O₃)₂]³⁻ in wastewater into more valuable material, is proposed in this work. Considera-

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tion of synthesis methods and study of the optical and photocatalytic activity of a nanocomposite with oxide NPs, especially TiO₂-Ag (Au, Fe), are a very important cytotoxicity test also for biosafety.

Therefore, this study aims to determine the effect of Ag on the surface structure, spectroscopic features (light irradiation at 488 nm) and cytotoxicity of Ag/TiO₂ series prepared by deposition method using silver nitrate and TiO(OH)₂ suspension.

2. EXPERIMENTAL

A raw material for obtaining the sample was a suspension of hydrated titanium dioxide TiO(OH)₂ (metatitanic acid), which is a product of the intermediate stage of processing of titanium concentrates and slags at the plant "Sumykhimprom". Nanosized particles of TiO₂ modified with silver were obtained in aqueous solutions of TiO(OH)₂ by adding alkali NaOH to form anatase modification with a range of silver concentrations from 0 to 8 wt. %. The number of samples: TiO₂, ATO4 (4 wt. % Ag), ATO8 (8 wt. % Ag). Mira 3 Tescan with EDX (Oxford INCA x-act) was used to study morphology and for elemental analysis. The particle shape and size were found with high-resolution TEM (JEM 1400). A transmission electron microscopy (TEM) study of the morphological features of nanoparticles was conducted. The samples of nanoparticles dried at room temperature were analyzed using a TEM JEM-1400 (Jeol, Japan) at an accelerating voltage of 80 kV. Electron diffraction of nanoparticles was performed on TEM with the same accelerating voltage, with the introduction of a field aperture and removal of an objective aperture. Raman spectroscopy spectra of powders (Horiba Jobin-Yvon T64000) were used to study the structural properties of silver-doped TiO₂ powders using an Ar-Cr laser at 488 nm. IR spectroscopic studies of the samples were performed using a Fourier spectrometer FSM-1201. The culture of MDCK (Madin-Darby canine kidney cell line) cells was obtained from L.V. Gromashevskogo Institute of Epidemiology and Infection Diseases of NAS of Ukraine (Kyiv, Ukraine). Cells were cultured in 45 % Dulbecco's Modified Eagle's Medium (DMEM, Sigma, USA) and 45 % RPMI-1640 (Sigma, USA) with 10 % (v/v) fetal bovine serum (FBS, Sigma, USA) and 100 U/ml gentamycin. Cell viability was assessed using an MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay after NPs exposure, since only viable cells have functional mitochondrial dehydrogenase enzymes that can reduce MTT to formazan [13]. The viability (%) of the treated cells was defined as the percentage of absorbance compared to control untreated cells (100 % viability). The percentage of cell viability under the condition of NPs action was calculated as in work [15].

3. RESULTS AND DISCUSSION

The morphology of TiO₂/Ag materials was analyzed by TEM and SEM. Fig. 1 shows that Ag NPs on a TiO₂ support are dispersed and deposited in a homogeneous layer. After loading Ag (4 wt. %), the sizes of TiO₂ particles decreased to 13-20 nm. "Ball-shaped" particles of silver with a developed crystalline structure in TiO₂

were observed. The results show that TiO₂ can be present in the tetragonal phase. Ag and TiO₂ have different work functions ($\Phi_{\text{TiO}_2} = 4.2 \text{ eV}$, $\Phi_{\text{Ag}} = 4.6 \text{ eV}$) and, hence, when silver is in contact with TiO₂, electrons will transfer from TiO₂ to silver. So, these electrons transfer to silver, and loads on the surface of silver will be scavenged by the electron acceptor, thus decreasing the recombination between electron and hole, thereby silver atoms act as electron traps. Thus, the existence of a silver atom in Ag/TiO₂ can facilitate the transport of more holes to the surface and enhance the optical and bactericidal activity. Ag particles on TiO₂ act as electron-hole separation centers.

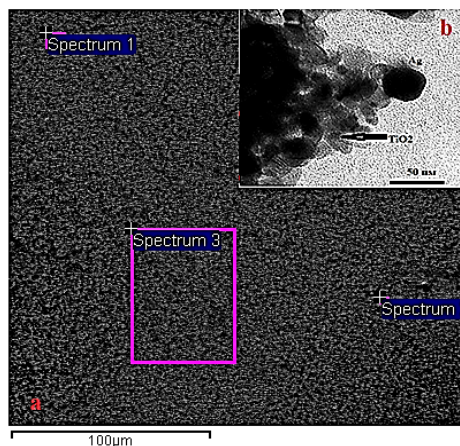


Fig. 1 – Structure of doped TiO₂: SEM (a) and TEM (b). Scale for the TEM image is 50 nm

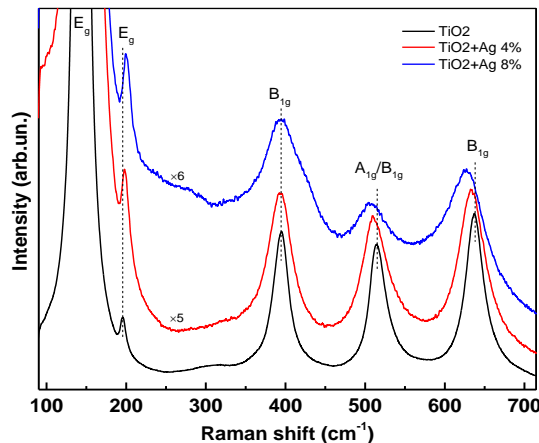


Fig. 2 – Raman spectra of TiO₂-Ag nanocomposites

There is a high-frequency shift and an increase in the half-width of the E_g (1) band at 142 cm⁻¹ and E_g (2) band at 196 cm⁻¹, while the B_{1g} band at 636 cm⁻¹ and A_{1g}/B_{1g} band at 513 cm⁻¹ show a low-frequency shift and a significant increase in the half-width in the Raman spectra of silver-doped nanopowders (Fig. 2). A wide complex band in the range of 220-300 cm⁻¹ is due to the processes of multiphonon scattering [16]. Since in the Raman spectra all oscillations move mainly oxygen atoms, the introduction of silver atoms changes the local coordination of oxygen around Ti⁴⁺. Doping with silver to maintain charge neutrality creates oxygen vacancies in the TiO₂ lattice. If the silver ion replaces the Ti⁴⁺ ion during doping, the bonds of the Ti-O-Ti

complex will be distorted and new bonds of Ag–O–Ti or Ag–O–Ag complexes will be formed. According to the calibration curve [17], we found that the average size of anatase crystallites for doped powders is 10 and 8 nm at a silver content of 4 and 8 wt. %, respectively. It is well known, doping with metal ions in an optimal concentration prevents the growth of nanocrystallites.

IR spectra analysis of the studied samples showed that the first sample was characterized by the manifestation of absorption bands of Ti–O oscillations with frequencies of ~ 504 and 664 cm^{-1} (Fig. 3, spectrum 1). For IR spectrum 2 of the sample, a shift of the absorption frequencies of Ti–O oscillations to the long wavelength direction was observed (Fig. 3, spectrum 2). The shift of characteristic oscillations of the Ti–O bond towards lower frequencies when modifying anatase with silver is caused by distortions of the symmetry of the coordination environment of Ti⁴⁺ and is a sign of the presence of silver cations in the crystal structure. A change in the shape of oscillations of the Ti–O absorption band and the manifestation of an absorption band with a frequency of $\sim 895\text{ cm}^{-1}$ characterize the distortion of the titanium-oxygen octahedron. Frequencies ~ 1110 and 1188 cm^{-1} characterize surface oscillations of functional groups (–OH) and are related to the doping method (Fig. 3, spectrum 2). There is a further change in the shape of the absorption bands for anatase due to loading of the sample with 8 wt. % of Ag (Fig. 3, spectrum 3). The content of hydroxyl groups in TiO₂ surface samples (IR analysis) is important for antimicrobial and photocatalytic properties because in the process of UV irradiation, OH groups on the defective surface of TiO₂ are active due to hole capture with the subsequent formation of $\cdot\text{OH}$ radicals for the destruction of toxic organic substances or pathogens. Besides, the presence of silver provides an efficient process of photogeneration of electrons, their transfer from the conduction band of Ag to TiO₂ with the subsequent formation of a Schottky barrier, which inhibits the rate of recombination of photogenerated charges.

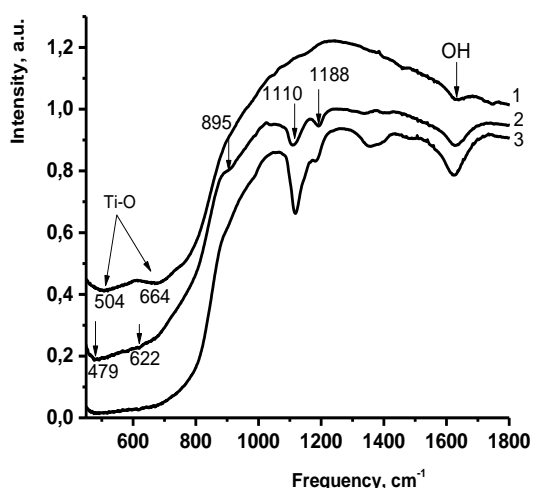


Fig. 3 – IR spectra of TiO₂ (1), TiO₂ + 4 % Ag (2) and TiO₂ + 8 % Ag (3) powders

EDX analysis of the synthesized samples also confirmed the presence of Ag. For example, the elements content: Ti – 46.27 wt. %, O – 46.69 wt. %, S – 0.14 wt. %, Ag – 3.3 wt. % (sample ATO4). There is also a certain amount of S as a dopant for high optical and photocatalytic activity, as in work [18].

Modern medicine begins to actively use nanotechnology in clinical diagnostics, targeted drug delivery, cancer treatment by hyperthermia, and other fields [13-15]. Nevertheless, the toxicity of nanostructured materials is an open issue due to several factors: high reactivity, intrinsic toxicity of the material, and non-specific interactions with biological objects, that are determined by particle shape, size and structure. Biocompatibility, toxicity and ability to penetrate cells are the main criteria that determine the effectiveness of NPs in medicine [17-19]. Determination of the cytotoxicity of synthesized titanium dioxide (TiO₂) with different percentages of argentum (Ag) is an integral component of any drug development process. The research was carried out using an MTT assay.

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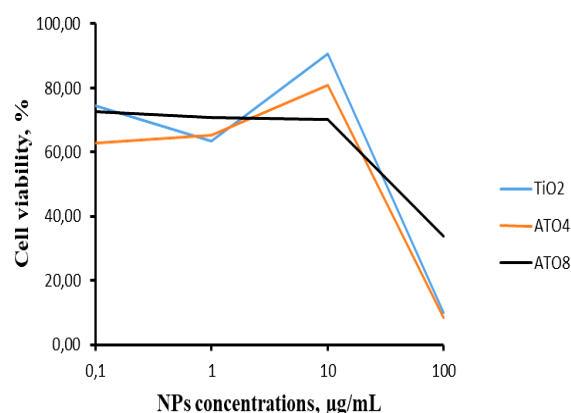


Fig. 4 – Viability of MDCK cells cultivated on different samples of TiO₂ and TiO₂ with Ag (ATO4 and ATO8) in glycerin + water

As shown in Fig. 4, the composition of TiO₂ without Ag, with 4 and 8 wt. % Ag in glycerin + water is less toxic to MDCK cells as compared to TiO₂ NPs in C₂H₅OH+1,3-propanediol. Thus, these samples do not decrease the cell viability at a concentration from 0.1 to 10 µg/ml. Inhibition of mitochondrial activity is detected only at a concentration of 100 µg/ml, the percentage of life ranged from 8 to 33 %. At the same time, for MDCK cells, the CC₅₀ index of TiO₂ diluted with C₂H₅OH+1,3-propanediol was 2.5 times lower compared to the glycerin + water suspension. The obtained results indicate lower toxicity of NPs in the glycerin + water suspension, regardless of the introduction of silver molecules in an amount of 4 or 8 wt. %, their CC₅₀ values were 50 and 3.9-58.5 µg/ml for MDBK and MDCK cells, respectively. MTT data [19] showed that Ag-doped TiO₂ NPs (synthesis from titanium isopropoxide) dose-dependently reduced the viable number of cells in the concentration range 25-200 µg/ml. Besides, cell viability decreases with increasing concentration of Ag dopant (> 4 %). On the other hand, pure TiO₂ NPs did not reduce the viability of HepG2 cells. So, oxidative stress could be one of the potential mechanisms of toxicity induced by Ag-doped TiO₂ NPs in human liver cancer HepG2 cells.

Thus, the generation of antimicrobial agents based on metal oxide TiO₂ doped with low concentrations of

noble elements is characterized by low toxicity and prolonged action and is most often used to create composite nanomaterials. Generally, this type of composite systems demonstrates a synergistic effect compared to the activity of individual oxides.

4. CONCLUSIONS

In summary, an experimental study of Ag/TiO₂ nanocomposite samples shows the following in the phase formation processes: carrying out redox reactions of oxidation of Ti (III) precursor species into TiO₂ (anatase), respectively, accompanied by the reduction of silver cations on the oxide surface in the form of silver clusters. The crystalline phase of Ag-TiO₂ was confirmed by IR and Raman analysis. The average size of pure and 8 wt. % Ag doped TiO₂ particles was determined to be 25-30 and 13-15 nm, respectively, using TEM images. The optical activity of Ag/TiO₂ with a shift of the mode E_g from 143 to 150 cm⁻¹ and FWHM from 12 to 19 cm⁻¹ was stated due to a decrease in

TiO₂ crystallites. The appearance of structural defects as a result of doping, which leads to a distortion of octahedra of the TiO₆ crystal structure, the occurrence of oxygen vacancies, Ti⁴⁺ ions and surface states (Raman, IR), must be accompanied by changes in radiative recombination due to changes in the electronic structure within the bandgap. As a result of cytotoxicity studies, it was shown that the type of solvent depends on the toxicity level of the studied NPs for cell cultures. Thus, it was determined that an increase in the concentration of silver leads to increased cytotoxicity for cell cultures. The results obtained made it possible to determine CC₅₀ values, which are the primary test for subsequent antiviral activities.

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Вплив Ag на поверхневий стан TiO₂, оптичну активність та його цитотоксичність

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Наночастинки (НЧ) Ag/TiO₂ було отримано простим методом хімічного осадження з використанням нітрату срібла та суспензії TiO(OH)₂. Частинок срібла осаджували як на поверхні, так і в самих частинках TiO₂ в залежності від концентрації Ag. Композити Ag/TiO₂ були охарактеризовані рентге-

нівською дифракцією, просвітлюючою електронною мікроскопією, скануючою електронною мікроскопією, раманівською та фотолюмінесцентною спектроскопією. Клітинна життєздатність оцінювалась за допомогою аналізу МТТ (3-(4,5-диметилтіазол-2-іл)-2,5-дифенілтетразолію бромід) після впливу НЧ. Показано оптичну активність Ag/TiO₂ зі зсувом моди E_g від 143 до 150 см⁻¹ та FWHM від 12 до 19 см⁻¹ внаслідок зменшення кристалітів TiO₂. Оптична активність зростає зі збільшенням концентрації Ag до 8 мас. %. У зоні контакту Ag-TiO₂ утворюється бар'єр Шоттки, що покращує розподіл заряду і таким чином уповільнює рекомбінацію фотогенерованих електронів та фотогенерованих дірок. Отримані результати свідчать про меншу токсичність НЧ у суспензії гліцерин + вода, незалежно від введення молекул срібла в кількості 4 або 8 мас. %, їх значення CC₅₀ становили 50 та 3,9-58,5 мкг/мл для клітин MDBK (нирки бика) та MDCK (нирки собаки) відповідно. Натомість НЧ TiO₂, розчинені в C₂H₅OH+ 1,3-пропандіолі при введенні молекул срібла, були значно більш токсичними для клітин MDBK у порівнянні з чистим TiO₂, їх значення CC₅₀ становили 6,5 та 4 мкг/мл. Одержані НЧ Ag/TiO₂ внаслідок їх оптичної активності будуть досліджені як протигрибковий матеріал для інгібування бактерій та вірусів у воді.

Ключові слова: Ag/TiO₂, Випромінювання, Раманівський, Дефекти, Оптична активність, Цитотоксичність, МТТ.