Investigation of biological and photocatalytic activity of multimodal nanopowders produced by pulsed electron beam evaporation in vacuum

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Abstract. Nanopowders doped with silver were produced by the method of pulsed electron beam evaporation. The pore sizes of the nanoparticles were 25-32 nm. Evaluation of photocatalytic and cytotoxic properties on cells was carried out. The prospects of using multimodal nanopowders as a photocatalytic agent, as well as for use in medicine, have been shown.

1. Introduction

Nanopowders doped with silver are interesting compounds for use in the field of medicine, due to antimicrobial and antitumor properties [1, 5]. Bismuth oxide, often acts as an object in the creation of photocatalysts, increased stability is created due to the silver coating, which nanoparticle have bactericidal properties. The main requirements determining the effectiveness of nanoparticles for use in the medical and pharmaceutical sphere are biocompatibility and non-toxicity.

Nanopowders were prepared by pulsed electron beam evaporation (PEBE) [2]. During the study were obtained nanopowders of bismuth oxide, zirconium oxide, with additives (1 wt.% Ag and 5 wt.% Ag) of silver nitrate.

The purpose of this work is to study the photocatalytic and biological activity of bismuth and zirconium oxide nanopowders doped with silver, to study the prospects for their possible use, including in nanomedicine.

2. Materials and methods

Nanopowders were prepared using the installation NANOBIM-2 [2]. The installation works as follows: the electron beam is focused in the hole of the upper gas-dynamic window, then passes through the second gas-dynamic window, is additionally focused by a deflecting coil on the target [2]. Under the influence of the pulsed electron beam, is evaporated the target material. The target was fired on air at the temperature 600°C, the pellets were compressed on a manual press (40 mm diameter and 15 mm height).The resulting steam plasma mixture is cooled by low-pressure gas in the evaporation chamber, where condensation and nanopowder (NP) formation occur. Then NP comes the collection system. Evaporation was carried out in the mode: accelerating voltage 38 kV, beam current 0.3 A, pulse duration 100 μs, frequency 50 Hz, evaporation time 45 minutes, pressure in the evaporation

chamber ~ 4 Pa. After that, the bismuth oxide nanopowder was annealed. Zirconium oxide was not annealed after preparation [3].

The method for evaluating the photocatalytic properties of composite powders consisted of the following: the methyl violet dye (MV) was dissolved in distilled water (10 μg/ml concentration); Then, an aqueous 300 μL suspension (for a 100 μg/ml NP concentration) consisting of 10 mg of NP and 5 ml of distilled water and 300 μL (for a 300 μg/ml NP concentration) consisting of 20 mg of NP and 5 ml of distilled water was added to the solution. The suspension was then irradiated on a UV gas discharge lamp DRS 250-3 for 40 minutes. To determine the rate of discoloration of the solution, its optical density was measured, for this purpose an aliquot of 4 ml of each solution was taken into a quartz cuvette and placed in an Ecros PE-5400UF spectrophotometer. Optical density measurements were performed at the wave length 595 nm before and after UV irradiation.

The cytotoxicity of silver-coated oxide nanomaterials was tested in human and animal cell cultures: on Vero green monkey cell culture and human tumor culture. HeLa Cytotoxicity was assessed by cell viability using an MMT test [4]. Studies were conducted at three concentrations (K): 0.1, 0.5 and 1 mg/ml. Cell cultures were placed in 96-well plates of 100 μl. Culturing was carried out for 24 hours. After which NP suspensions were added to the wells, after settling for 2 hours, the environment was drained and DMSO dye was added. After that, a study was conducted on the flatbed scanner TecanInfinite M200 PRO.

3. Results and discussions

After obtaining the bismuth oxide nanopowder, the initial bismuth oxide NP (sample S0) was annealed in alund crucibles at 200, 300, 500 and 750°C, hereinafter referred to as samples S200, S300, S500 and S750, respectively. A color change was established when heating NP bismuth oxide in the sequence: brown → yellow → red (cherry) → yellow. The isothermal holding time is 10 minutes, cooling was carried out together with the furnace to 100-150°C. The textural properties of the NP were studied by the BET method on the analyzer MicromeriticsTriStar 3000 V6.03 A. The pore sizes of the NP were 25-32 nm, volume 0.069-1.121 cm^3/g, specific surface area (SSA) of the target 1.4 m^2/g, SSA of samples 10-23 m^2/g. The NP of zirconium oxide is a mesoporous material according to the IUPAC classification, since the pore diameter of this NP ranges from 23.8 to 37.9 nm. Obtained NP have low specific surface area. If to compare ZrO_2-1%Ag and ZrO_2-5%Ag, then it is possible to see that at bigger concentration of silver the surface area and volume of a time are less, than at ZrO_2-1%Ag, but at the same time at ZrO_2-5%Ag the diameter of pores is more. The same method was used to obtain NP CaF_2 and BaF_2 to study the cytotoxic effect on cells. CaF_2 (1) has a pure composition, and CaF_2 (2) was doped with Mn, Yb, Si, Er similarly to the previous NP.

Table 1. Value of the coefficient *k* for NP Bi_2O_3.

Sample Bi_2O_3	Concentration 100 (μg/ml)		Concentration 300 (μg/ml)	
	Absolute value	Lead to control	Absolute value	Lead to control
Control	-0.0249	1	-0.0152	1
S0	-0.0295	1.18	-0.0264	1.74
S200	-0.0186	0.74	-0.0198	1.30
S300	-0.0208	0.84	-0.0265	1.75
S500	-0.0184	0.74	-0.0216	1.42
S750	-0.0198	0.80	-0.0159	1.04

The dependence of the discoloration rate of the MV solution on the time of exposure to UV radiation can be described by the linear equation $y = kx + b$. The value of the photodegradation rate (photodegradation) is determined by the tangent of the inclination angle of the line $y = kx + b$ (i.e., coefficient k), with which it is possible to approximate the resulting curves as a result of photodegradation of the dye (reference points corresponding to the optical density measured at certain intervals). The higher the coefficient, the faster the solution is discolored. The results are shown in tables 1-3.

Table 2. Value of the coefficient k for NP Bi_2O_3 + Ag, 1 wt.%.

Sample (Bi_2O_3 + Ag, 1 wt.%)	Concentration 100 (μg/ml)		Concentration 300 (μg/ml)	
	Absolute value	Lead to control	Absolute value	Lead to control
Control	-0.0134	1	-0.0134	1
S0	-0.0139	1.03	-0.0261	1.9
S200	-0.0076	0.57	-0.016	1.19
S500	-0.0077	0.57	-0.0086	0.64

Table 3. Value of the coefficient k for NP Bi_2O_3 + Ag, 5 wt.%.

Sample (Bi_2O_3 + Ag, 5 wt.%)	Concentration 100 (μg/ml)		Concentration 300 (μg/ml)	
	Absolute value	Lead to control	Absolute value	Lead to control
Control	-0.0148	1	-0.0146	1
S0	-0.0246	1.66	-0.031	2.12
S200	-0.0178	1.20	-0.0156	1.06
S300	-0.0156	1.18	-0.031	2.12
S400	-0.0175	1.18	-0.0168	1.15
S500	-0.0149	1.01	-0.0126	0.86

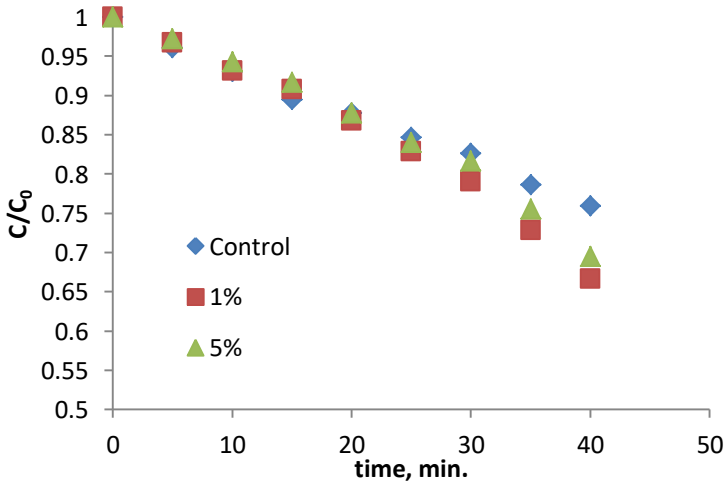

Figure 1. Results of the given values of photocatalytic properties NP ZrO_2-1%Ag and ZrO_2-5%Ag.

According to tables 1-3, bismuth oxide samples S0 and S300 at the concentration 300 μg/ml were found to be better among all measured. High activity is caused by annealing of samples, which made it possible to reduce the defect of the nanopowder structure, its coating with silver, as well as an increase in the suspension concentration.

According to the results of the experiment on photocatalistic activity (figure 1), it can be seen that the nanopowder ZrO_2-1% Ag and ZrO_2-5%Ag showed photocatalistic properties, and it can be seen that the powder ZrO_2-1%Ag showed stronger photocatalistic properties than ZrO_2-5%Ag, this is due to the fact that the nanopowder ZrO_2-1%Ag has a larger surface area of particles.

An experiment to study the cytotoxic properties of NP zirconium oxide was performed on the culture of African green monkey kidney cells (Vero) and human cervical carcinoma cells (HeLa) [4].

The results of the biological activity of the bismuth oxide nanopowder are shown in tables 4, 5.

Table 4. Effect of NP Bi_2O_3 on relative cell culture viability HeLa.

Sample		S200			S300			S500		
K (mg/ml)	Test	0.1	0.5	1	0.1	0.5	1	0.1	0.5	1
		Bi_2O_3								
	100	97.3 ±3.9	79.4 ±8.1	74.5 ±4.5	99.4 ±4.5	83.2 ±5.9	67.2 ±7.3	97.8 ±5.6	73.7 ±4.4	72.1 ±5.9
		Bi_2O_3 + Ag, 5 wt.%								
Relative viability (%)	100	95.7 ±2.9	76.8 ±5.1	64.9 ±1.6	92.1 ±6.0	70.2 ±6.0	50.0 ±2.0	92.0 ±7.5	71.6 ±4.1	48.1 ±1.8
		Bi_2O_3 + Ag, 1 wt.%								
	100	86.3 ±9.8	96.8 ±6.1	92.3 ±7.4	123.1 ±9.5	97.1 ±3.4	80.0 ±6.6	101.0 ±4.1	85.0 ±4.8	93.4 ±4.7

Table 5. Effect of NP Bi_2O_3 on relative cell culture viability Vero.

Sample		S200			S300			S500		
K (mg/ml)	Test	0.1	0.5	1	0.1	0.5	1	0.1	0.5	1
		Bi_2O_3								
	100	87.7 ±4.5	53.8 ±2.6	39.5 ±2.3	84.1 ±2.4	49.8 ±3.3	36.6 ±1.0	78.1 ±5.4	38.4 ±3.2	33.7 ±2.6
		Bi_2O_3 + Ag, 5 wt.%								
Relative viability (%)	100	74.8 ±6.0	46.7 ±2.0	34.6 ±2.8	47.7 ±2.9	39.4 ±1.4	40.4 ±0.9	54.9 ±4.2	33.8 ±2.5	35.9 ±2.5
		Bi_2O_3 + Ag, 1 wt.%								
	100	86.3 ±9.8	96.8 ±6.1	92.3 ±7.4	123.1 ±9.5	97.1 ±3.4	80.0 ±6.6	54.9 ±4.2	33.8 ±2.5	35.9 ±2.5

It was revealed that all samples of NP Bi_2O_3 have cytotoxic effect on cages of tumoral and not tumoral origin. At the same time, an increase in the annealing temperature and the presence of silver

enhance the cytotoxic effect of NP, both individually and jointly. It is worth noting Bi_2O_3 + Ag, 1 wt.%, nanopowder affects tumor cells (kills more than 60%).

Results of biological activity of zirconium oxide nanopowder are shown in figures 2 and 3.

According to the results of the study, it was revealed that the sample NP ZrO_2 does not have a cytotoxic effect on non-neoplastic Vero cells, regardless on the silver concentration (figure 3), but it can be observed that NP ZrO_2 acts on tumor cells HeLa at any silver concentration. In figure 2, it can be seen that for sample ZrO_2-1%Ag, tumor cell viability decreased by 18-23% as compared to control at concentrations of 0.1 mg/ml and 0.5 mg/ml. For the ZrO_2-puge sample, cell viability decreased only at the concentration 1 mg/ml by 20% compared to the control. The best result was obtained for NP ZrO_2-5%Ag at the concentration 1 mg/ml, in which case the viability of tumor cells decreased by 35% compared to the control.

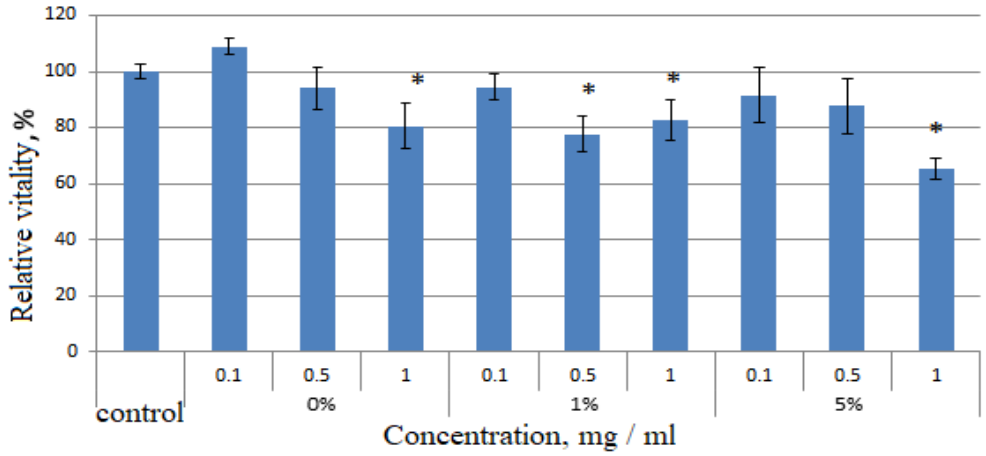

Figure 2. Experiment results NP ZrO_2 for cells HeLa.

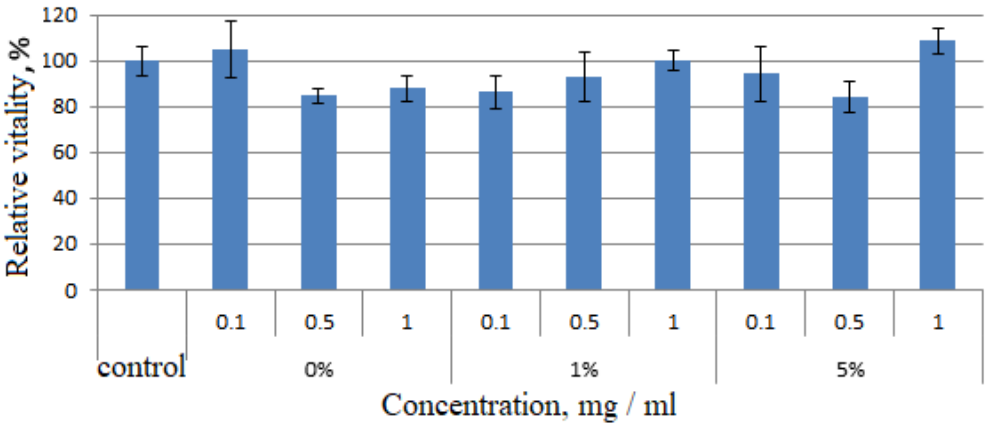

Figure 3. Experiment results NP ZrO_2 for cells Vero.

The results of the biological activity of fluoride nanopowder are shown in figures 4 and 5.

According to the results of the study, it was revealed that the sample NP CaF_2(1) does not have a cytotoxic effect on non-neoplastic Vero cells, regardless on the silver concentration (figure 4), but it can be observed that NP CaF_2(1) acts on tumor cells HeLa at any silver concentration. In figure 4, it can be seen that for sample CaF_2(1), tumor cell viability decreased by 40-42% as compared to control at concentrations of 1 mg/ml and 0.5 mg/ml; for sample CaF_2(2), tumor cell viability decreased by 38-42% as compared to control at concentrations of 1 mg/ml and 0.5 mg/ml; for sample BaF_2 has a very strong toxic effect on both Vero and Hela cells.

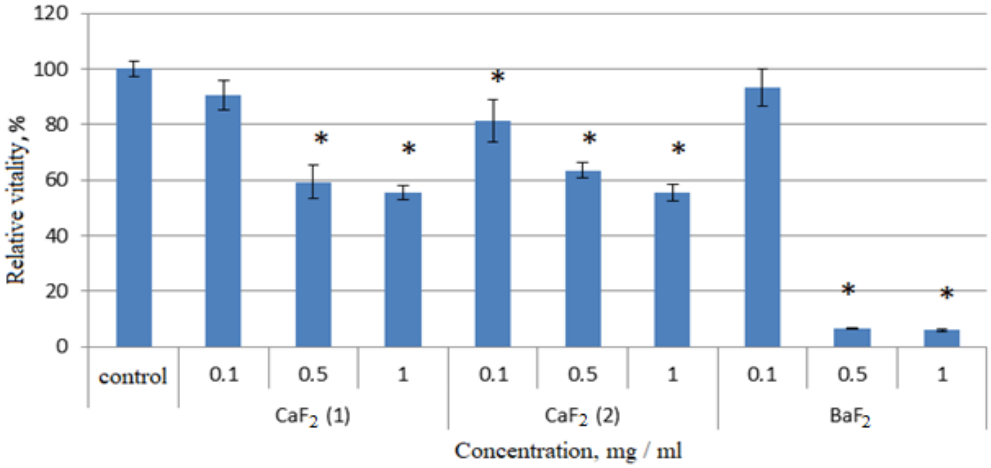

Figure 4. Experiment results NP fluorides for cells HeLa.

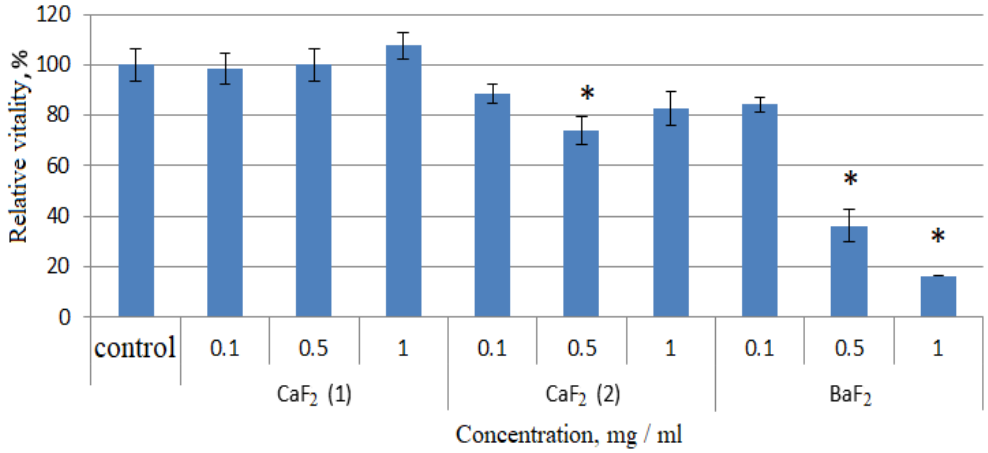

Figure 5. Experiment results NP ZrO_2 fluoridesfor cells Vero.

Thus, according to the results of the experience, it can be seen that NP zirconium oxide and CaF_2(1), if introduced into tumor cells, is a promising object for research in medicine.

4. Conclusion

Silver-doped bismuth and zirconium oxide nanopowders have been obtained.

All silver doped bismuth oxide nanopowder samples have cytotoxic effects on tumor and non-tumor cells. At the same time, the presence of silver enhances the cytotoxic effect of NP. At concentrations of 0.5 and 1 mg/ml, the viability of tumor cells decreases by 25-30% compared to the control, in non-tumor cells there is a decrease in viability at all concentrations by 30-65%.

Analysis for cytotoxic properties showed that NP ZrO_2-1%Ag and ZrO_2-5%Ag and CaF_2(1) did not affect non-neoplastic cells, in the case of tumor cells, the nanopowders studied showed a decrease in the viability of tumor cells from 18 to 35%.

Using an experiment on photocatalytic activity, it was found that the studied nanopowders proved to be photocatalysts.

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