doi: 10.56761/EFRE2024.C5-O-010604

Biological properties of zinc oxide and barium fluoride nanoparticles after nanosilver coating and annealing

A.S. Gerasimov[1,2,], V.G. Ilves[2], S.Yu. Sokovnin[1,2], T.R. Sultanova[3], M.V. Ulitko[1,4]*

[1]*Ural Federal University, Yekaterinburg, Russia*
[2]*Institute of Electrophysics, UB, RAS, Yekaterinburg, Russia*
[3]*Institute of Immunology and Physiology UB RAS, Yekaterinburg, Russia*
[4]*Institute Of Medica Cell Technology, Yekaterinburg, Russia*
[*]*andrei.gerasimov@urfu.ru*

Abstract. This paper presents a study of the biomedical application of zinc oxide (ZnO-Zn) and barium fluoride (BaF_2) nanoparticles (NPs), including after annealing and coating with nanosilver obtained by pulsed electron evaporation. The work describes the cytotoxicity and antioxidant activity of NPs against the Hep-2 cell line, and antibacterial properties against Escherichia coli bacteria. The loading capacity of the NPs has been studied. It was found that the coating with nanosilver in most cases enhances the samples activity. The optimal annealing and doping conditions for each of the tasks have been identified. The principal possibility of using NPs to create drug delivery systems was pronounced.
Keywords: nanoparticles, zinc oxide, barium fluoride, cytotoxicity, loading capacity.

1. Introduction

Nanobiotechnology is rapidly developing field of research with various applications in the biomedical and pharmaceutical industries [1]. Recently, nanoparticles (NPs) of metal oxides and fluorides of various elements have been of great interest due to their unique physical, chemical and biological properties [2]. These properties allow them to serve as antioxidants [3], agents for antitumor therapies [4], targeted drug delivery systems [5], etc.

NPs have a great potential in biomedical field, but for the successful application of these agents in practice, it is necessary to fully understand their validity. The aim of this work is the investigation of the bioactivity of zinc oxide and barium fluoride NPs, as well as the study of such factors as thermal annealing and nanosilver coating.

In this work, the NPs of zinc oxide (ZnO-Zn) and barium fluoride (BaF_2) obtained by pulsed electron evaporation method (PEBE) were studied. The cytotoxicity and antioxidant activity of NPs in human laryngeal carcinoma Hep-2 cell culture were analyzed. The antibacterial activity of NPs against gram-negative bacteria Escherichia coli, as well as their loading capacity, has been studied.

2. Materials and method

2.1. NPs' characterization

The NPs were produced by the PEBE method in vacuum [6]. This method allows to obtain NPs with small size of grains, high specific surface area and strong non-stoichiometry. This ensures a high reactivity of the obtained samples. In addition, the feature of this method is the formation of a significant number of defects of various types. The obtained samples of ZnO-Zn and BaF_2 were fine-grained aggregates of particles combined into agglomerates with a specific surface area of 29.17 and 19.01 m^2/g, respectively. The parameters of the studied frequencies were presented in more detail earlier [6].

The radiation-chemical method for the preparation of nanosilver-coated composites based on ZnO-Zn and BaF_2 was performed by analogy with the work [7]. A suspension based on an AgNO3 sorbitol solution with the addition of iron oxide NPs was irradiated in Petri dishes using a nanosecond electron accelerator URT-0.5 (IEP UB RAS) [8]. The irradiated suspensions were kept for 96 hours, then the solutions were drained, and the resulting powders were washed with distilled water three times and dried.

The annealing of NPs samples, including their composites, was carried out in electrocorundum crucibles at temperatures from 0 to 400 °C. Annealed samples will be further designated S0 – S400 depending on the annealing temperature. The isothermal exposure time was 10 minutes, the samples were cooled in an oven to temperatures of 100–150 °C.

2.2. NPs' biomedical application assay

The cytotoxic and antioxidant activity of ZnO-Zn and BaF_2 NPs, including annealed and coated with nanosilver, was studied on the human laryngeal carcinoma Hep-2 cell line. Antibacterial activity – against gram-negative bacteria Escherichia coli. NPs' suspensions were administrated into cell cultures at concentrations of 0.5 mg/ml and 1 mg/ml. A minimum concentration of 0.1 mg/ml was also used for the study on Hep-2 culture. Oxidative stress was modeled by introducing hydrogen peroxide into cell cultures at a final concentration of 0.5 mM.

To assess the viability of cells, the MTT test was used, a colorimetric test to assess the metabolic activity of cells. The cells were seeded into a 96-well tablet at a dose of 1×10^5 cl/ml. The volume of the suspension was 100 µl. The cells were cultured 24h at 37 °C in an Igla MEM ("Biolot") medium containing glutamine (1%), 10% embryonic veal serum and gentamicin (50 mg/l), in a humidified atmosphere of 5% CO_2. After that, suspensions of NPs were added to the wells in appropriate concentrations. 15 holes were used as a control sample.

To assess the loading capacity of bismuth oxide and calcium fluoride NPs, the antitumor antibiotic Doxorubicin (lyophilizate, manufacturer Pharmfhemi B.V., the Netherlands) was used.

The introduction of drugs was carried out by suspending 10 mg of zinc oxide and barium fluoride NPs in 5 ml of an aqueous solution of the drug Doxorubicin (1 mg/ml). Then, the suspended samples were separated by centrifugation (4000 rpm, 10 min), washed in distilled water and dried for 3 days. To assess the concentration of the loaded drug (C_{st}, mg/ml), the supraventricular part was examined by spectrophotometric method at a wavelength of $\lambda = 490$ nm, corresponding to the maximum absorption of doxorubicin. The dried samples were re-suspended in 1 ml of distilled water and centrifuged, after which the filler liquid was examined on a spectrophotometer using a technique similar to that of estimating the mass of the loaded drug. Based on the obtained concentrations of the released drug (C_d, mg/ml) and the volume of the initial suspensions, the mass of the introduced drug (m_d, mg) and the loading capacity (LC) of NPs were calculated.

3. Results and discussion

3.1. Cytotoxicity and antioxidant activity of NPs against Hep-2 cell culture

The result of the study of the viability of human laryngeal carcinoma cell lines Hep-2, after administration of the studied NPs in various concentrations, is shown in Table 1. It is noted that all the studied samples had a cytotoxic effect against the considered tumor line.

Analyzing the obtained data, the greatest cytotoxic effect can be identified for the samples ZnO-Zn S0 and ZnO-Zn+Ag S200 at a concentration of 1 mg/ml and BaF_2+Ag S400 at a concentration of 0.5 mg/ml. For these samples, a decrease in the viability of tumor cells by more than 80% was recorded. At the same time, no direct correlation was found between the annealing temperature and cytotoxicity. In general, nanosilver coating enhances the cytotoxic effect.

Antioxidant activity has been investigated in relation to the ability of NPs to inhibit hydrogen peroxide. The results of the study for samples of low frequency ZnO-Zn and BaF_2 are shown in Fig. 1 and 2, respectively. Annealing of the ZnO-Zn NPs up to 400 °C allowed to increase the antioxidant activity. The administration of the ZnO-Zn S200 sample did not lead to a significant increase in the viability of Hep-2 cells, which is consistent with the detected high cytotoxicity of this sample.

Table 1. Relative viability of Hep-2 cells after NPs' administration.

Sample	Concentration, mg/ml		
	0.1	0.5	1
ZnO-Zn S0	89.13 ± 7.45	74.22 ± 0.8*	15.14 ± 1.43*
ZnO-Zn+Ag S0	35.07 ± 3.05*	32.4 ± 1.7*	56.07 ± 13.98
ZnO-Zn+Ag S200	42.81 ± 2.54*	24.68 ± 2.67*	17.77 ± 1.9*
ZnO-Zn+Ag S400	88.79 ± 18.17	24.75 ± 1.52*	60.18 ± 22.18
BaF_2 S0	109.61 ± 5.56	28.87 ± 2.2*	27.11 ± 1.23*
BaF_2 S200	97.65 ± 3.0	66.37 ± 3.93*	20.64 ± 1.35*
BaF_2 S400	100.15 ± 2.81	84.93 ± 9.34	40.12 ± 8.81*
BaF_2+Ag S0	91.22 ± 13.23	30.64 ± 0.83*	29.41 ± 3.14*
BaF_2+Ag S200	115.51 ± 8.76	31.92 ± 1.72*	41.92 ± 2.25*
BaF_2+Ag S400	79.57 ± 7.32*	17.63 ± 0.46*	28.39 ± 3.0*

* – the difference with the control is valid ($p < 0.05$)

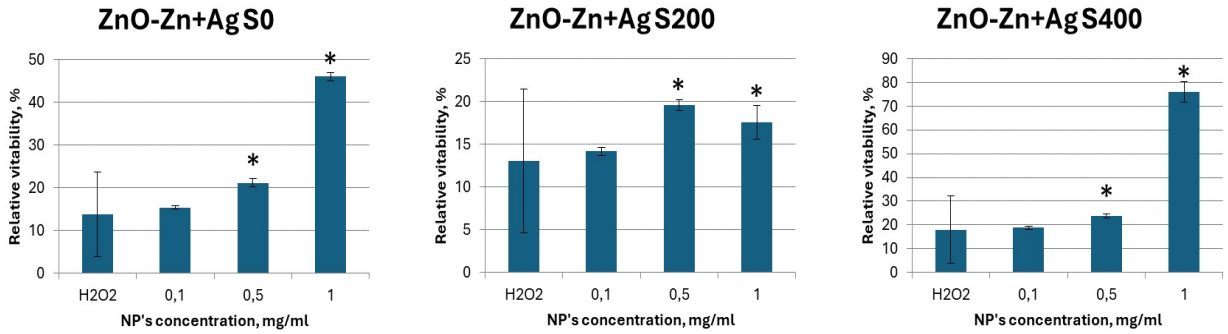

Fig. 1. Relative Hep-2 cell viability after hydrogen peroxide and ZnO-Zn+Ag NPs administration (* – the difference with the control with peroxide is valid ($p < 0.05$)).

Nanosilver coating with BaF_2 nanosilver made it possible to increase the antioxidant activity of these samples. Nevertheless, the BaF_2 S200 and BaF_2+Ag S400 samples did not show pronounced antioxidant ability, which may be due to their high cytotoxicity.

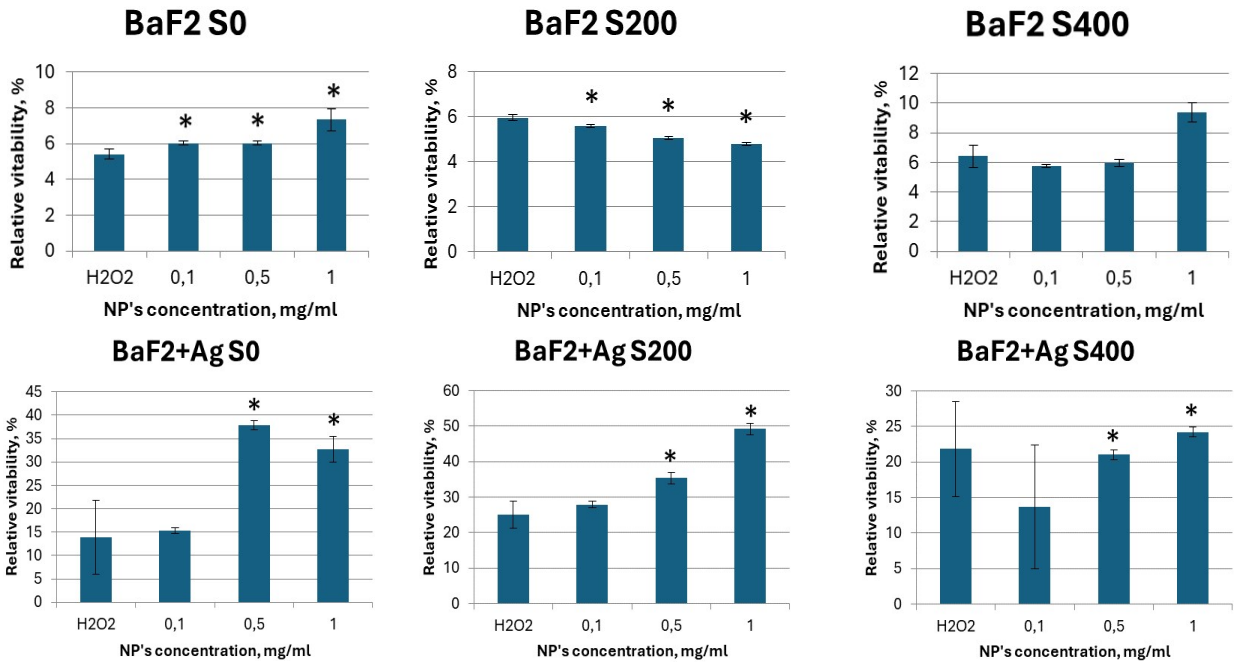

Fig. 2. Relative Hep-2 cell viability after hydrogen peroxide and BaF_2 и BaF_2+Ag NPs administration (* – the difference with the control with peroxide is valid ($p < 0.05$)).

Thus, by selecting the treatment conditions of the NPs: doping and annealing temperatures, it is possible to create the most suitable material for a specific task. When developing antitumor agents, the samples of ZnO-Zn S0, ZnO-Zn+Ag S200 and BaF_2+Ag S400 NPs should be considered. To select the most powerful antioxidant compound, ZnO-Zn S0, BaF_2+Ag S0 and BaF_2+Ag S200 are most suitable.

3.2. Antibacterial activity of NPs

Samples of zinc oxide and barium fluoride nanopowders showed significant antibacterial activity against the suspension culture of Escherichia coli bacteria (Table 2).

Table 2. Relative viability of Escherichia coli bacterial culture after NPs' administration.

Sample	Concentration, mg/ml	Viability	
		Absorbance	Relative to control, %
BaF_2 S0	0.5	0.45 ± 0.01	57.7
	1	0.40 ± 0.00	51.3
BaF_2 S200	0.5	0.36 ± 0.03	46.2
	1	0.34 ± 0.02	43.6
BaF_2 +Ag S0	0.5	0.27 ± 0.01	34.6
	1	0.25 ± 0.00	32.1
BaF_2 +Ag S200	0.5	0.21 ± 0.00	26.9
	1	0.24 ± 0.01	30.8
ZnO-Zn	0.5	0.36 ± 0.03	46.2
	1	0.33 ± 0.01	42.3
ZnO-Zn+Ag S0	0.5	0.25 ± 0.01	32.1
	1	0.20 ± 0.01	25.6
ZnO-Zn+Ag S200	0.5	0.27 ± 0.03	34.6
	1	0.26 ± 0.02	33.3

Barium fluoride inhibits the growth of E.coli by 42.3–69.2%, zinc oxide – by 53.8–66.7%. Nanosilver coating enhances the antibacterial effect of nanopowders. Dose dependence (5 or 10 mg/ml) was not detected.

3.3. Drug-loading capacity of NPs

To assess the loading capacity of the ZnO-Zn and BaF_2 NPs, a comparison of the released volume of the drug was performed. The results of the study are shown in Table 3.

Table 3. The loading capacity of the NPs in relation to the drug "Doxorubicin".

Sample	C_{st}, mg/ml	C_d, mg/ml	m_d, mg	LC
Control	1.00	–	–	–
Dox-BaF_2 S0	1.031 ± 0.005	0.166 ± 0.002	0.83 ± 0.01	0.083 ± 0.001
Dox-BaF_2 S200	0.992 ± 0.003	0.181 ± 0.001	0.91 ± 0.01	0.091 ± 0.001
Dox-BaF_2+Ag S0	1.074 ± 0.007	0.093 ± 0.002	0.47 ± 0.01	0.047 ± 0.001
Dox-BaF_2+Ag S200	1.129 ± 0.029	0.100 ± 0.001	0.50 ± 0.01	0.050 ± 0.001
Dox-ZnO-Zn	1.005 ± 0.006	0.157 ± 0.002	0.79 ± 0.01	0.079 ± 0.001
Dox-ZnO-Zn+Ag S0	1.056 ± 0.011	0.090 ± 0.001	0.45 ± 0.00	0.045 ± 0.000
Dox-ZnO-Zn+Ag S200	1.074 ± 0.007	0.086 ± 0.001	0.43 ± 0.00	0.043 ± 0.000

Samples of zinc oxide and barium fluoride NPs demonstrated a loading capability, which shows the fundamental possibility of their use to create drug delivery systems. The BaF_2 NPs showed a slightly higher loading capacity than the ZnO-Zn. Annealing of samples increases the loading capacity due to the purification of the NPs from impurities, but the nanosilver coating reduces.

4. Conclusion

The conducted research revealed the prospects of using ZnO-Zn and BaF_2 NPs in biomedical technologies. It was shown that NPs treatment significantly affects the manifested properties. Thus, the NPs ZnO-Zn+Ag S200 showed significant cytotoxicity against the human laryngeal carcinoma cell line Hep-2, while the sample ZnO-Zn+Ag S400 showed significant antioxidant activity. By selecting the parameters of NP processing in this way, it is possible to expand the possibilities of nanobiotechnology, in particular in the field of cancer therapy.

Pronounced antibacterial properties of NP zinc oxide and bismuth fluoride and their composites were found. Moreover, sliver doping enhances the antibacterial activity. It is also noted that studied samples can be used to create drug delivery systems. The NPs' loading capacity increases after thermal annealing.

Additional research is needed to determine the exact conditions of NPs treatment, under which the properties shown will be most optimal. Increasing the potential of the studied NPs will help the development of technologies in the biomedical field.

Acknowledgement

This work was financially supported by the Russian Science Foundation (project No 22-19-00239).

5. References

[1] S. Sabir, M. Arshad, and S. K. Chaudhari, Zinc oxide nanoparticles for revolutionizing agriculture: synthesis and applications, *The scientific world journal*, vol. **2014**, 925494, 2014; doi: 10.1155/2014/925494

[2] D. Bharathi, V. Bhuvaneshwari, Synthesis of zinc oxide nanoparticles (ZnO NPs) using pure bioflavonoid rutin and their biomedical applications: antibacterial, antioxidant and cytotoxic activities, *Research on Chemical Intermediates*, vol. **45**, 2065, 2019; doi: 10.1007/s11164-018-03717

[3] X. Ge, Z. Cao, and L. Chu, The antioxidant effect of the metal and metal-oxide nanoparticles, *Antioxidants*, vol. **11**(4), 791, 2022; doi: 10.3390/antiox11040791

[4] J.J. Xu, et al., Metal nanoparticles as a promising technology in targeted cancer treatment, Drug Delivery, vol. **29**(1), 664, 2022; doi: 10.1080/10717544.2022.2039804

[5] R. Liu, et al., Advances of nanoparticles as drug delivery systems for disease diagnosis and treatment, *Chinese chemical letters*, vol. **34**(2), 107518, 2023; doi:10.1016/j.cclet.2022.05.032

[6] S. Sokovnin, V. Ilves, *Nanopowders of Metal Oxides and Fluorides: Preparation, Properties, and Applications*, Jenny Stanford Publishing, 2023.

[7] M.E. Balezin, S.Y. Sokovnin, and M. Uimin, Preparation of iron oxide nanopowders by the radiation-chemical method, *Journal of Physics: Conference Series*, vol. **2064**(1), 012086, 2021; doi: 10.1088/1742-6596/2064/1/012086

[8] S.Y. Sokovnin, M.E. Balezin, Improving the Operating Characteristics of an URT-0.5 Accelerator, *Instruments and Experimental Techniques*, vol. **48**, 392, 2005; doi: 10.1007/s10786-005-0068-0