

ANTIVIRAL PROPERTIES OF CERIUM NANOPARTICLES

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Abstract

According to the results of the study, the threshold limit values (TLVs) of CeO₂ and Ce nanoparticles (NPs) for the culture of swine kidney embryonic cell line (SKECL) have been estimated. The TLVs are 0.1 µg/cm³ for Ce NPs, and 1 µg/cm³ for CeO₂ NPs. All NPs were non-toxic for white mice at concentration of 2000 mg/kg, which opens up a perspective for the further use of these NPs in the development of antiviral substances. CeO₂ and Ce NPs have decreased the titer of *Teschovirus* by 1.46–2 lg₁₀ TCD₅₀/cm³ at TLVs at all stages of virus reproduction. Sensitivity of the strain Dniprovskiy-34 of species *Teschovirus* A, serotype *Porcine teschovirus* 1 (PTV-1), to solvents, proteolytic enzyme trypsin under the presence of CeO₂ and Ce NPs has been studied. Under the presence of solvents, and trypsin, NPs decreased the infectious titer of the virus slightly by only 0.23 lg₁₀ TCD₅₀/cm³. CeO₂ and Ce NPs have not shown antiviral activity under heating to 50 °C, either with the addition of 1 M solution of MgCl₂ or without it, and did not cause significant decrease of infectious activity of the virus under pH values 2.0, 7.2, and 11.0 within 10 minutes exposition. Ability of CeO₂ and Ce NPs to interact with viral particles, change their morphology, which can affect infective activity of the virus significantly, has been shown. Addition of CeO₂ and Ce NPs to the antigen has caused the decrease of the titer of virus-neutralizing antibodies, though it remained high. The results of the studies can be used in development of disinfectants, and antiviral drugs.

Keywords: cerium nanoparticles, *Teschovirus* A, cerium dioxide, antiviral activity, virus inactivation, electron microscopy

INTRODUCTION

The search for new substances with antiviral properties, and development of new antiviral drugs based on nanoparticles (NPs) is one of the most important tasks of virology at the beginning of XXI century. Virologists in the whole world constantly screen substances with antiviral activities, create vectors for their delivery, and develop new antiviral drugs with targeted action (Lozovskiy *et al.*, 2014; Lokshyn *et al.*, 2014; Derevianko *et al.*, 2019; Mazurkova *et al.*, 2010). Modern scientific achievements in the field of nanotechnology open up vast perspectives for the production, and use of metal NPs, which can exist in forms of oxides,

hydroxides, colloidal compounds, hydrated forms, citrate-stabilized forms, and forms that are stabilized by other stabilizing agents (nanoaquachelates).

Use of biogenic metals in form of NPs opens up for the humanity not only new perspectives for the development of new efficient antiviral drugs, but also creates new risks, because NPs, being biologically active substances, exhibit cytotoxic activity (Derevianko *et al.*, 2019).

A lot of data on the antiviral activity of cerium dioxide NPs (CeO₂ NPs) has become available in past two decades, and the number of publications is still rising. Thus, it has been proven that cell cultures L929, and EPT have developed antiviral resistance

against *Indiana vesiculovirus*, if previously treated with CeO₂ NPs at concentrations 2.0–10.0 µg/cm³ (Zholobak *et al.*, 2010). These results open up perspective for deeper investigation of water suspensions of CeO₂ NPs. Mechanisms of action, and biological effects CeO₂ NPs are considered promising for deeper studies.

In another study it has shown that CeO₂ NPs are highly efficient adjuvant and antiviral substance. They significantly enhanced the antiviral activity of IFN-CDNPs nanobiocomposite, which consisted of interferone, and CeO₂ NPs. CeO₂ NPs showed high efficacy while being used as an antiviral agent in white mice infected with *Herpes simplex virus* type 1 enhancing the efficiency of immune system *in vivo* (Shidlovska *et al.*, 2018).

CeO₂ NPs, synthesized by green method, has been found to have antiviral activity against sabin-like poliovirus (type 1) in RD, and Hep-2C cell lines (Mohamed *et al.*, 2020). However, the exact mechanisms of CeO₂ NPs antiviral activity against sabin-like poliovirus are yet to be understood.

CeO₂ NPs has also been discussed as having potential protective activity against SARS-CoV-2 in some review studies (Ulker, Abacioglu and Sehirli, 2021).

In study by Neal *et al.* (2021) authors found that silver-modified CeO₂ NPs (AgCNPs) inactivate human coronavirus OC43, and human rhinovirus 14, and has been shown to be promising potential disinfectants (Neal *et al.*, 2021).

Nefedova *et al.* (2022) found that CeO₂ NPs with positive, and negative surface charge have significant antiviral activity against SARS-CoV-2, influenza virus A/WSN/1933, transmissible gastroenteritis virus, bacteriophage φ6, whereas they were ineffective against picornavirus EMCV, and negatively charged CeO₂ NPs were slightly active against bacteriophage MS2 (Nefedova *et al.*, 2022). The authors have tested also Ce³⁺ ions, and SiO₂ NPs, and has reached a conclusion that antiviral activity of CeO₂ NPs is not due to the release of Ce³⁺ ions or non-specific effects caused by the nano-size of particles. Thus, the mechanisms of the antiviral activity of CeO₂ NPs are yet to be understood, and the knowledge on the mechanisms of antiviral activity of other nanoparticles can shed the light on them.

High potential of CeO₂ NPs as antiviral agents has led to the development of a number of methods of their obtaining, which can be applied in industry (Shcherbakov *et al.*, 2011; Lokshyn *et al.*, 2017). Along with these methods medical preparations based on CeO₂ NPs (Spivak *et al.*, 2014a), and substances, which can be used as preparations (Spivak *et al.*, 2014b) were developed. However, these substances, and preparations are not available commercially at the moment.

Amongst other NPs, which antiviral activity is well studied are silver NPs (Ag NPs). They have shown to have broad spectrum antiviral activity against viruses from *Retroviridae*, *Hepadnaviridae*,

Paramyxoviridae, *Herpesviridae*, *Poxviridae*, *Orthomyxoviridae*, and *Arenaviridae* genera (Lu *et al.*, 2008; Elechiguerra *et al.*, 2005; Sun *et al.*, 2008; Baram-Pinto *et al.*, 2009; Rogers *et al.*, 2008).

The mechanisms of the antiviral activity of Ag NPs are studied better than those of other NPs. Ag NPs are thought to prevent the entrance of the pathogen to the cell (Galdiero *et al.*, 2014), and are shown to suppress the initial stages of DNA replication of HIV-1 via prevention of the interaction between gp120 glycoproteins, and CD4 receptors, and, subsequently, the contact of the pathogen with the cell (Elechiguerra *et al.*, 2005).

Other possible mechanisms of Ag NPs antiviral activity can include interactions between silver NPs, and:

- capsids, and/or viral surface proteins with further neutralization of pathogen's biological activity;
- biomembranes' components leading to the prevention of the virus's contact with the cell, and its entrance to cytoplasm;
- viral genome;
- viral, and cell factors that are essential for the replication (Rai *et al.*, 2014).

This information can be used to understand mechanism of antiviral activity of other NPs.

Nanosized structures of other metals, such as gold, iron, and titanium, are also known to exhibit antiviral activity. There are reports on the activity of titanium dioxide (TiO₂) NPs against influenza virus. Thus, virucidal activity of TiO₂ NPs with size 4–10 nm, which were obtained by the hydrolysis of titanium tetrachloride (TiCl₄), against influenza virus strain H₃N₂, which was cultured in suspension of chicken embryo cells culture, has been shown (Mazurkova *et al.*, 2010). According to data from transmission electron microscopy, TiO₂ NPs have destroyed the virus within 30 min incubation. Researchers have suggested that antiviral activity of NPs can be explained by the direct contact of NPs with viral particles, and further destruction of capsids (Mazurkova *et al.*, 2010).

Hence, studying antiviral effects of CeO₂, and Ce NPs on reproduction of picornaviruses is topical, has a valuable scientific, and practical importance.

MATERIALS AND METHODS

Objects and Methods of Studies

Objects of studies were: strain Dniprovskiy-34 of species *Teschovirus* A (TV-A), serotype *Porcine teschovirus* 1 (PTV-1); CeO₂ NPs; citrate-stabilized Ce NPs; transmittable cell culture of embryonic swine kidney cell line (SKECL), white outbred mice, rabbits.

Strain Dniprovskiy-34 of TV-A species, serotype PTV-1 was obtained from the collection of the Institute of agricultural microbiology and agro-industrial production of NAAS of Ukraine.

CeO₂ NPs were obtained from Danylo Zabolotny institute of microbiology and virology of NAS of Ukraine, from doctor of biological sciences, professor Spivak M. Ya. CeO₂ NPs were synthesized from CeCl₃ by chemical synthesis (Shcherbakov *et al.*, 2016).

Citrate-stabilized Ce NPs were obtained in "Nanomaterials and nanotechnologies" LTD., from doctor of technical sciences, Kaplunenko V. H. Citrate-stabilized Ce NPs were produced by method of erosion-explosive dispersion (Kosinov and Kaplunenko, 2007).

Transmittable cell culture of embryonic swine kidney cell line (SKECL) was obtained from Institute of experimental and clinical veterinary medicine of NAAS of Ukraine.

Methods

For studying the cytotoxicity of NPs water suspensions of NPs were diluted to needed concentration, and their pH was neutralized before use. Further, suspensions were autoclaved under the pressure 0.6 atm during 20 min. Peptone water (PW), peptone agar (PA), thioglycol medium, and Sabouraud agar were used to control sterility. Method of direct inoculation was used. Only if the media remained clear within 14 days, suspensions were used for further studies.

Cytotoxicity of NPs was measured in SKECL cell culture. Samples of culture medium with the addition of studied substances at different concentrations were added to the monolayer of cells. Double dilutions of NPs were used. 4 test tubes containing cell culture were used for each concentration. In control, change of culture medium was also performed, but the NPs were not added.

Measurement of the results was performed within 7 days period. Monolayer of cells was studied for signs of cytotoxic effects of substances via optical microscopy: disruption of the integrity of monolayer; appearance of spherical, wrinkled, and degenerated (signs of vacuolization, granularity, flattening) cells. Level of toxicity was evaluated by 4-cross system, in which each cross corresponded to degeneration of 25% of the area of cells monolayer.

Acute toxicity *in vivo* was evaluated using white outbred mice. The acute toxicity was evaluated via the method of limit-test, according to Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure, OECD Guidelines for the Testing of Chemicals, Section 4 (Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure, 2008).

This method allows to establish basic parameters of substances toxicity: median lethal dose, and its standard error ($LD_{50} \pm \text{std.err}$) for substances with unknown toxicity and if LD_{50} is higher than 2000 mg/kg – to observe the development or absence of clinical signs of toxicity.

According to data on mortality that was obtained in the study, the values of LD_{50} were calculated. If 3 or less animals have perished, the LD_{50} is thought to be less than 2000 mg/kg, so the exact value of LD_{50}

has to be measured by another method. If 3 or more animals have survived, the LD_{50} is thought to be more than 2000 mg/kg.

Refreshment, maintenance, and accumulation of the strain Dniprovskiy-34 was performed using SKECL cell culture via common methods. Accumulation of virus was performed in cell culture, which was cultivated in cultural vials or in plastic flasks. Before the introduction of the virus, the culture medium was poured out, and the monolayer of cells was washed with saline solution or Hanks' salts solution. 0.5, and 1 cm³ of virus-containing suspension were added to containers, and stored at 37 °C within 1 h for contact. After the contact, the monolayer was washed with Hanks' salts solution, covered with maintaining medium, and left in thermostat. At 75% of cytopathic action (CPA) the cultures were frozen three times at the temperature -18 °C.

Titration of viruses was performed in transmittable cell culture in test tubes with observation of cytopathic effect (CPE), and evaluation of 50% tissue cytopathic dose (TCD_{50}/cm^3). For the evaluation of TCD_{50} , a series of the dilutions of the virus in culture medium for cells has been prepared. Every dilution of the virus at volume 0.1 cm³ was used to infect cells in 4 test tubes, which subsequently were incubated in thermostat at 37 °C within 7 days. Assessment of CPA was performed on 4, and 7 days. The titer of the virus has been calculated via Reed's and Muench's method (1938).

NPs were screened, and selected according to their ability to reduce virus-induced CPE in animal cell culture. First, the antiviral activity of NPs has been studied at their TLVs. If NPs caused reduction of the virus titer by 2.0 lg TCD_{50} or more, then therapeutic index (TI) was calculated. For this purpose, minimum active concentration (MAC) was calculated. MAC is the concentration at which the substance decreases the infectious titer of the virus by 1.25–1.5 lg TCD_{50} as compared to the titer of the virus in control. TI was calculated as TLV to MAC ratio.

Initial screening was conducted in animal cell culture according to the next plot: 1:10 dilution of native virus-containing suspension (1 to 8) was added to culture medium containing studied substances at TLV, after which 0.1 cm³ of the obtained suspension was added to the monolayer of cells.

To determine the mechanisms of antiviral action, different plots of NPs introduction into the monolayer of cells, which allow to influence different stages of virus reproduction, have been used:

1. Prophylactic plot – 1.0 cm³ of NPs at TLVs were added to test-tubes containing a monolayer of cells, and exposed for 24 h. After this, the solution was removed, and 1.0 cm³ of 1 to 8 ten-fold dilution of the virus-containing suspension were added to the same test-tubes.
2. Treatment plot – 1.0 cm³ of 1 to 8 ten-fold dilution of the virus-containing suspension were added

to test-tubes with a monolayer of cells. After exposure for 1.5 h previously applied at various concentrations virus-containing suspension was removed from the cell culture, and 1.0 cm^3 of the studied NPs at TLVs were added to test-tubes with cell culture.

3. Virucidal plot – exposure of native virus-containing suspension, and double TLVs of the studied NPs was maintained for 24 h at a ratio of 1:1. Then 1.0 cm^3 of 1 to 8 ten-fold dilution of the resulting suspension were added in a test tubes containing a monolayer of cells.

The study of biological, and physicochemical properties of viruses under the action of NPs was carried out according to the following plots:

- sensitivity of viruses to solvents (chloroform, and ether);
- proteolytic enzyme trypsin;
- thermal resistance;
- stability of viruses at different pH values.

Resistance of viruses to chloroform was determined by the method of Bogel and Mayr (1961), to ether by the method of Rovozzo and Burke (1973).

The virus-containing cultural suspension, which has or does not have NPs at TLVs, was centrifuged at 3000 g for 30 min. The supernatant was mixed with chloroform at a ratio of 1:1, shaken for 1 h at room temperature, and kept at 4°C for 16 h. The mixture was then centrifuged at 3000 g, and the supernatant was collected.

The ethyl ether at a ratio of 1:4 was mixed with the virus-containing suspension, which has or does not have NPs at TLVs, shaken, and kept for 18 h at 4°C , stirring the mixture periodically. Removal of ether from the virus-containing suspension was performed at 37°C for 30 min.

The conclusion about the influence of NPs on the properties of viruses was made by the difference of titers of viruses treated with lipid solvents, and control.

Sensitivity to trypsin was determined by the method of Matheka and Mayr (1962).

For this purpose, a 0.5% solution of trypsin was added to the viral suspension, in which there are or are not NPs at TLVs at a ratio of 1:1, periodically shaken, and kept in a thermostat at 37°C for 2 h. The susceptibility of the virus to trypsin under the influence of NPs was determined by the difference between the titers of the virus in the experimental, and control samples.

Thermal resistance of viruses was determined by the method of Rovozzo and Burke (1973), and stabilization by Mg^{2+} ions was determined by the method of Melnick and Wallis (1962).

The thermal resistance of the virus was studied by heating samples in which there are or are no NPs at TLVs in a heated bath for 1 h at 50°C in the presence of 1 M MgCl_2 , and without it. All virus samples were titrated. The difference in virus titers was used to

conclude that they were thermally resistant under the influence of NPs.

The stability of viruses under the influence of NPs at different pH values of the medium was determined by the method of Mayr *et al.* (1977).

For this purpose, the viruses were kept in a medium with a pH value of 2.0; 7.2; 11.0 at room temperature for 10 min. Then in all samples the pH was adjusted to 7.2. The sensitivity of the virus to acidic, and alkaline pH values was determined by the difference between virus titers.

In the first stage, the viruses were concentrated, and purified with chloroform, and ammonium sulfate. Purification of the virus with chloroform was performed according to the method of Bogel K. and Mayr (1961). To do this, the virus-containing culture suspension was centrifuged at 3000 g for 30 min. The supernatant was collected. 1 part of chloroform was added to 2 parts of the selected virus-containing suspension, and homogenized for 30 min. Then it was kept for 12–18 h at 4°C , then centrifuged at 3000 g for 20 min. Ammonium sulfate was then added to the supernatant to 50% saturation, and the suspension was kept at 4°C for 1 h. The precipitate was separated by centrifugation at $5\text{--}10^\circ\text{C}$ for 20 min at 3000 g, resuspended in 0.9% saline, and dialyzed against 0.9% saline for 16–18 h.

Further purification, and concentration of viruses was performed by ultracentrifugation on a preparative ultracentrifuge VAC-601 according to the method of Mainor (1988) in our modification.

The virus-containing suspension was purified in a stepwise sucrose density gradient (15–45%) in Tris-HCl buffer with pH 7.4 by centrifugation at 100,000 g for 4 h at 4°C . After that, 5 fractions were selected. Control of the purity of virus samples was performed using transmission electron microscopy.

Transmission electron microscopy of viruses, citrate-stabilized Ce, and CeO_2 NPs was performed both in the Institute of Agricultural Microbiology Agro-industrial Production of NAAS and in the Danylo Zabolotny Institute of Microbiology and Virology of NAS of Ukraine.

Transmission electron microscopy of viruses, citrate-stabilized Ce, and CeO_2 NPs, and viral particles under the action of NPs was performed on collodion grids with carbon spraying. The samples were contrasted with a 2% solution of phosphotungstic acid (pH 7.0) for 2 min. The study of virus preparations was performed on a Czechoslovak electron microscope Tesla BS540 at an instrumental magnification of 22,000, and an accelerating voltage of 75 kV.

The morphology of citrate-stabilized Ce, and CeO_2 NPs, and viral particles under the action of NPs was studied using a transmission electron microscope JEOL JEM-1400 (Japan). Diluted suspensions of NPs, and virus-containing suspensions were applied to copper mesh with a carbon-formvar coating, and dried in air at room temperature. Samples were contrasted either with 1% uranium acetate solution for 30 s or with 2% phosphotungstic acid solution

I: Scheme of immunization of rabbits

No.	Way of administration	Presence of adjuvant	Total content of immunogen
1.	Subcutaneously	+	1 mg of protein/2 cm ³
2.	Intradermally alongside spinal cord to 8–10 locations (within 7 days)	–	1 mg of protein/1 cm ³
3.	Subcutaneously (within 3 days)	+	1 mg of protein/2 cm ³
4.	Intradermally alongside spinal cord to 8–10 locations (within 4 days)	–	1 mg of protein/1 cm ³
5.	Subcutaneously (within 3 days)	+	1 mg of protein/2 cm ³

for 20 s. The finished samples were examined at an accelerating voltage of 100 kV. The internal structure of the NPs (amorphous or crystalline) was determined with electron diffraction in Diffraction mode with the introduction of the field, and the output of the objective aperture of the transmission electron microscope.

Hyperimmune blood sera to the TV-A strain Dniprovskiy-34 of serotype PTV-1 were obtained in rabbits. Selected after quarantine, and previously individually marked animals were divided into groups by random selection using a table of random numbers. Prior to the introduction of the studied antigens with substances, the experimental animals were acclimatized for 5 days in a vivarium for testing. Then they were weighed, and injected with test substances.

Purified and concentrated virus-containing suspension of strain TV-A Dniprovskiy-34 was used for hyperimmunization of rabbits. Montanide ISA 25 adjuvant (SEPPIC, France) was added to the portion of the finished antigen used for subcutaneous administration in the recommended ratio (Volkova *et al.*, 2011).

Immunization of rabbits was performed according to the scheme presented in Tab. I.

Selection was performed 7–14 days after the last immunogen administration.

To detect virus-neutralizing antibodies in the sera of animals in the neutralization reaction used 100 TCD₅₀ antigen, and 10 neutralizing doses of serum. 0.5 cm³ with 0.1 cm³ of 10 neutralizing doses of serum was added to the 0.5 cm³ virus with a content of 0.1 cm³ of 100 TCD₅₀ antigen. After keeping the resulting mixture at a temperature of 37 °C for 60–90 min, 0.2 cm³ was added to 2–4 tubes, in which 0.8 cm³ of support medium was previously added. Control, and test tubes were incubated at 37 °C. The results were recorded for 4–5, and 7–8 days.

Neutralization of the virus in these doses indicates the presence of antibodies in the test material.

When comparing the infectious activity of virus strains, a different Student method was used to determine the significance of the difference between the two compared indicators. To do this, we compared two independent samples (X_1), and (X_2):

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{\sum_{i=1}^n x_{i1}^2 - \frac{(\sum_{i=1}^n x_{i1})^2}{n_1} + \sum_{i=1}^n x_{i2}^2 - \frac{(\sum_{i=1}^n x_{i2})^2}{n_2}}{n_1 n_2 \left(1 - \frac{2}{n_1 + n_2}\right)}}}, \quad (1)$$

where:

n_1 the sample size of the first group;

n_2 the sample size of the second group;

\bar{X}_1 ... the sample mean of the first group;

\bar{X}_2 ... the sample mean of the second group (Gerald, 2018).

Obtained t -values were compared with the table of critical values of t . The calculations were performed in Microsoft Office Excel software.

RESULTS

Studies on the Morphology and Structure of Nanoparticles

Morphology and Structure of Citrate-stabilized Ce Nanoparticles

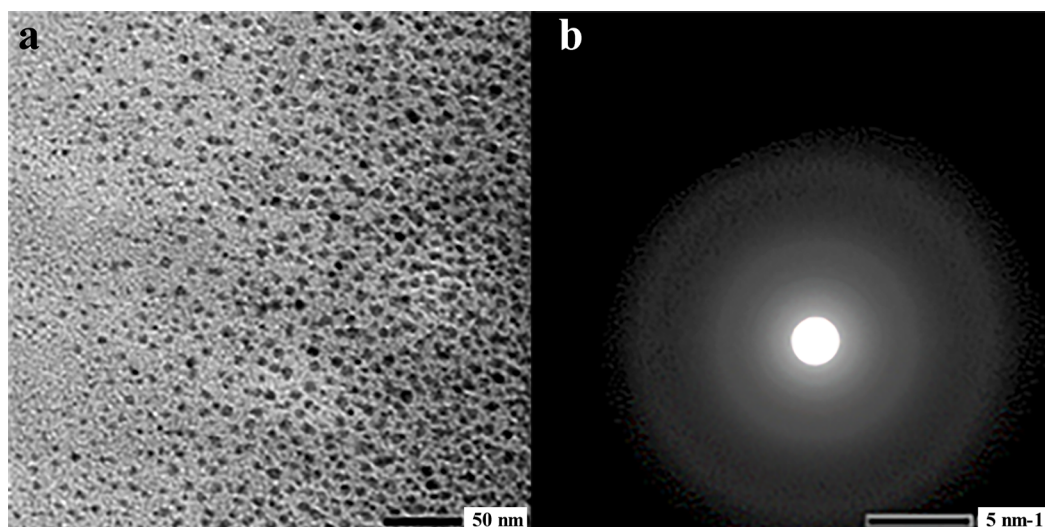
According to the results of transmission electron microscopy, it has been found that samples of citrate-stabilized Ce NPs contain separate NPs with sizes from 3 to 12 nm (Fig. 1a). Citrate-stabilized Ce NPs had an amorphous structure, which is confirmed by the corresponding electron diffraction patterns (Fig. 1b).

Morphology and Structure of CeO₂ NPs

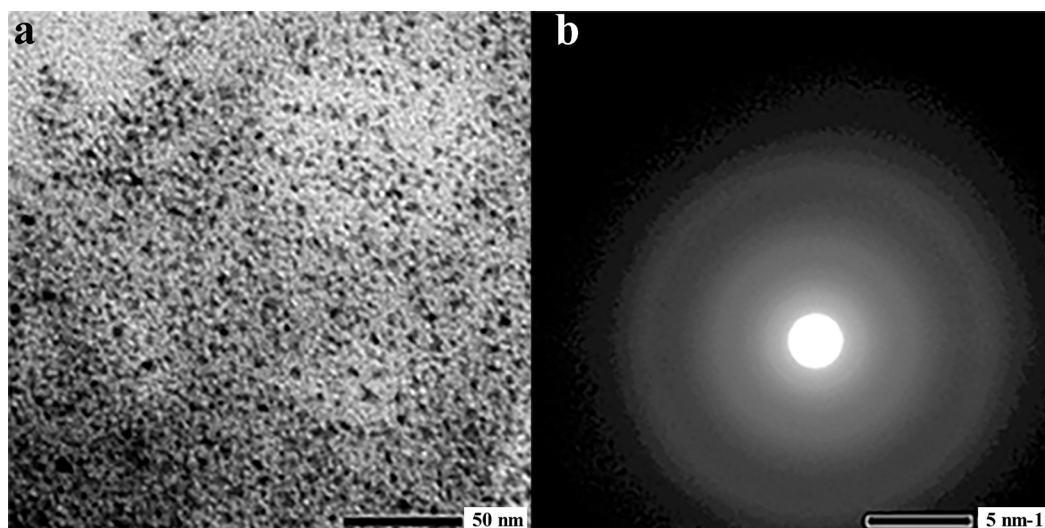
Transmission electron microscopy of CeO₂ NPs preparations has revealed that they contain discrete spherical NPs ranging in size from 3 to 12 nm (Fig. 2a). Electron diffraction pattern revealed amorphous structure (Fig. 2b).

Cytotoxicity of Nanoparticles in Cell Culture

Studies on the evaluation of TLVs of NPs have been conducted in SKECL cell culture. It was established that TLVs are 0.1 µg/cm³ for Ce NPs, and 100 µg/cm³ for CeO₂ NPs. In further studies in cell culture, these NPs were used at TLVs.



1: Transmission electron microphotographs of a Ce NPs sample: a – Ce NPs, b – electron diffraction pattern



2: Transmission electron microphotographs of a CeO₂ NPs sample: a – CeO₂ NPs, b – electron diffraction pattern

Evaluation of the Toxic Effects of Nanoparticles in Animals

Acute toxicity has been evaluated in accordance with Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure, OECD Guidelines for the Testing of Chemicals, Section 4, which is described above. This method allowed finding out the main parameters of substances toxicity, namely: the median lethal dose, and its standard error ($LD_{50} \pm \text{std.err}$) for substances with unknown toxicity, and if LD_{50} was higher than 2000 mg/kg, observing the development or absence of clinical signs of toxicity.

NPs were found being non-toxic for white outbred mice at a concentration of 2000 mg/kg.

Thus, we have found that the studied NPs were non-toxic for white mice at a concentration of 2000 mg/kg and TLVs of the studied substances were evaluated in SKECL cell culture.

Antiviral Activity of Ce and CeO₂ NPs According to the Treating-and-prophylactic Plots

Refreshment of TV-A strain Dniprovskiy-34 has been performed in SKECL cell culture through 3 passages. The accumulation of the virus was carried out in SKECL cell culture that was grown in culture vials and plastic flasks in quantities sufficient for research. Before use, the virus-containing suspension has been purified with chloroform. The virus titer was 6.0–6.23 lg TCD₅₀/cm³.

Studies on the antiviral activity of NPs according to the prophylactic plot have been conducted in SKECL cell culture (Tab. II). It was established that CeO₂, and Ce NPs showed prominent antiviral activity against TV-A strain Dniprovskiy-34, reducing its titer by 2.00 lg TCD₅₀/cm³. Next, the minimum active concentration of CeO₂, and Ce NPs has been measured in order to calculate their TI. It has been found that both CeO₂, and Ce NPs have TI 2. The results are presented in Tab. II.

II: Antiviral activity of NPs according to the prophylactic plot against TV-A strain Dniprovskiy-34 in SKECL cell culture

No.	Substance	TLV, $\mu\text{g}/\text{cm}^3$	Titer of virus, $\lg \text{TCD}_{50}/\text{cm}^3$	Difference between virus titers, $\lg \text{TCD}_{50}/\text{cm}^3$	TI
1	CeO ₂ NPs	1	4.00 ± 0.14	2.00	2
2	Ce NPs	0.1	4.00 ± 0.14	2.00	2
Control (no NPs)		–	6.00 ± 0.18	–	–

III: Antiviral activity of NPs according to the treatment plot against TV-A strain Dniprovskiy-34 in SKECL cell culture

No.	Substance	TLV, $\mu\text{g}/\text{cm}^3$	Titer of virus, $\lg \text{TCD}_{50}/\text{cm}^3$	Difference between virus titers, $\lg \text{TCD}_{50}/\text{cm}^3$	TI
1	CeO ₂ NPs	1	4.50 ± 0.14	1.50	2
2	Ce NPs	0.1	4.50 ± 0.14	1.50	2
Control (no NPs)		–	6.00 ± 0.16	–	–

IV: Virucidal activity of nanoparticles against TV-A strain Dniprovskiy-34 in SKECL cell culture (exposure 24 h)

No.	Substance	TLV, $\mu\text{g}/\text{cm}^3$	Titer of virus, $\lg \text{TCD}_{50}/\text{cm}^3$	Difference between virus titers, $\lg \text{TCD}_{50}/\text{cm}^3$	TI
1	CeO ₂ NPs	1	4.77 ± 0.16	1.46	2
2	Ce NPs	0.1	4.23 ± 0.14	2.00	2
Control (no NPs)		–	6.23 ± 0.12	–	–

The Antiviral Effect of Ce and CeO₂ NPs According to the Treatment Plot

The antiviral activity of NPs against the TV-A strain Dniprovskiy-34 has been evaluated in the SKECL cell culture according to the treatment plot (Tab. III).

CeO₂ and Ce NPs showed prominent antiviral activity and reduced the virus titer by $1.50 \lg \text{TCD}_{50}/\text{cm}^3$. MACs of CeO₂ and Ce NPs have been used to calculate TIs. It has been established that CeO₂ and Ce nanoparticles have TI 2 (Tab. II).

Virucidal Activity of Nanoparticles

Virucidal activity of NPs has been studied. The results of the studies are presented in the Tab. IV.

It has been found that CeO₂ and Ce NPs show prominent virucidal activity, decreasing the titer of the virus by $1.46\text{--}2.00 \lg \text{TCD}_{50}/\text{cm}^3$. It has been determined that CeO₂ and Ce NPs have TI 2.

Thus, the reproduction of viruses under the influence of NPs has been studied. It was established that CeO₂ and Ce NPs exhibit prominent antiviral activity at all stages of virus reproduction.

Sensitivity of Teschoviruses to Solvents (Chloroform and Diethyl Ether) Under the Influence of Nanoparticles

The sensitivity of teschoviruses to chloroform, and diethyl ether under the influence of CeO₂ and Ce NPs has been studied in SKECL cell culture (Tab. V). It was established that CeO₂ and Ce NPs caused a slight decrease in the virus titer by $0.23 \lg \text{TCD}_{50}/\text{cm}^3$.

Sensitivity of Teschoviruses to the Proteolytic Enzyme Trypsin Under the Influence of Nanoparticles

Antiviral activity of NPs against TV-A strain Dniprovskiy-34 under the influence of trypsin has been evaluated in SKECL cell culture (Tab. V).

CeO₂ and Ce NPs has been shown to cause a slight decrease of the infectious titer of the virus by only $0.23 \lg \text{TCD}_{50}/\text{cm}^3$.

Effects of Nanoparticles on Teschoviruses' Resistance to Heat

In the culture of SKECL cells, the thermal resistance of strain TV-A Dniprovsky-34 was determined by heating for 1 h at a temperature of 50 °C in the presence of 1 M MgCl₂ and without it (Tab. V).

It was found that CeO₂ and Ce NPs did not show high virucidal activity and did not affect the thermal resistance of TV-A strain Dniprovskiy-34. Thus, when the virus was heated at 50 °C without 1 M MgCl₂, the virus titer in all experimental variants, and controls was significantly reduced by $1 \lg \text{TCD}_{50}/\text{cm}^3$. In the presence of 1 M MgCl₂, the infectious titer of the virus did not change, which indicates the stabilization of the virus by Mg²⁺ ions and is characteristic to teschoviruses.

Determination of the Stability of Teschoviruses at Different pH Values Under the Action of Nanoparticles

The biological activity of TV-A strain Dniprovskiy-34 at pH 2.0, 7.2, and 11.0 was

V. Effects of nanoparticles on biological, and physicochemical properties of TV-A strain Dniprovskiy-34

No.	NPs	TLV, µg/cm ³	Infectious activity under the influence of biological, and physicochemical factors, lg TCD ₅₀ /cm ³									
			chloroform	diethyl ether	trypsin	control	heated			pH 2.0	pH 7.2	pH 11.0
							with MgCl ₂	without MgCl ₂	with MgCl ₂			
1	2	3	4	5	6	7	8	9	10	11	12	
1	CeO ₂ NPs	1	6.00±0.16	6.00±0.16	6.00±0.16	6.00±0.16	6.46±0.12	5.46±0.12	6.46±0.14	6.46±0.14	6.46±0.14	6.46±0.14
2	Ce NPs	0.1	6.00±0.16	6.00±0.16	6.00±0.16	6.00±0.16	6.46±0.12	5.46±0.12	6.46±0.12	6.46±0.12	6.46±0.12	6.46±0.12
Control (no NPs)			6.23±0.12	6.23±0.12	6.23±0.12	6.23±0.12	6.46±0.12	5.46±0.12	6.46±0.12	6.46±0.12	6.46±0.12	6.46±0.12

determined in the culture of SKECL cells; at room temperature, and an exposure of 10 min under the action of NPs (Tab. V).

CeO₂ and Ce NPs did not show virucidal activity at either neutral, alkaline or acidic pH values.

Concentration and Purification of Viruses

The accumulation of the virus was carried out in the culture of SKECL cell line, which was grown in culture vials, and flat flasks in an amount sufficient for research. It was determined that the virus titer was 6.46 lg TCD₅₀/cm³ (Tab. VI).

The infectious activity of the viruses did not change after purification with chloroform. The titer of the virus increased on average by 0.5 lg TCD₅₀/cm³ after concentrating with ammonium sulfate. The titer of the virus increased by 1.0–2.0 lg TCD₅₀/cm³ after purification and concentration by ultracentrifugation. The virus was distributed over the entire gradient, which is indicated by the infectivity of all selected fractions. The pronounced peak of infectious activity was detected in one fraction with a sucrose content of 30% and was 8.46 lg TCD₅₀/cm³ (Tab. VI).

For the control of virus purification quality, electron microscopy was done. Separated viral particles with size 25–28 nm (Fig. 3a), and other small objects, which can be structural elements of cells, were found in samples of TV-A strain Dniprovskiy-34, which were purified with chloroform, and concentrated with ammonium sulfate.

After the concentration, and purification in sucrose density gradient, 5 fractions were selected from each sample (supernatant, fractions with 15, 30, and 45% of sucrose respectively, and resuspended precipitate).

There were no viral particles in supernatant. There were small spherical, and irregular structures with size 3–7 nm in the fraction containing 15% of sucrose. There were no viral particles in studied samples (Fig. 3b).

Spherical viral particles with size 27–30 nm without external lipid envelope, and with specific to teschoviruses contrasting were found in the fraction containing 30% of sucrose (Fig. 3c, 3d). There were no structural components of cells, and no extraneous microorganisms.

Spherical virions with size 29–30 nm and spherical, and irregular objects with size 60–66 nm were found in the fraction, which contained 45% of sucrose (Fig. 3e, 3f).

Thus, the fraction containing 30% of sucrose has been selected for the further studies, because it contained viral particles with sizes 27–30 nm without external lipid envelope, and with specific for teschoviruses contrasting. Extraneous microorganisms, viral particles with disrupted integrity, partially contrasted (“empty”) viral particles, visible irregularly shaped fragments of viral particles, segments of capsids, structural elements of cells, and so on were absent. The titer of the virus was the highest.

VI: Infectious activity of strain TV-A Dniprovskiy-34 in cell culture SKECL

No.	Characteristics of samples	Titer of virus, lg TCD ₅₀ /cm ³	Difference in titer of virus as compared to control, lg TCD ₅₀ /cm ³
1	Virus-containing cultural suspension	6.46 ± 0.12	0
2	Virus, purified with chloroform	6.46 ± 0.12	0
3	Virus, concentrated with ammonium sulfate	6.96 ± 0.10	0.5
4	Supernatant after sedimentation with ultracentrifuge	4.46 ± 0.10	-2.0
5	Fraction with 15% content of sucrose	6.46 ± 0.12	0
6	Fraction with 30% content of sucrose	8.46 ± 0.10	2.0
7	Fraction with 45% content of sucrose	7.46 ± 0.10	1.0
8	Resuspended precipitate	6.46 ± 0.12	0

Interaction of Dniprovskiy-34 Strain Viral Particles With NPs

Interaction With Ce NPs

It was found that Ce NPs could significantly change the morphology of viral particles. Fig. 4a, 4b, 4c shows the viral particle with the adsorbed NPs, which in shape, and size corresponds to the Ce NPs, while the morphology of viral particle is unchanged. The size of the viral particles was 25–30 nm (Fig. 4a, 4b, 4c). Fig. 4a shows electron micrograph, which is made using uranyl acetate as contrasting agent, while Fig. 4b shows electron micrograph, made using phosphotungstic acid as contrasting agent. Both methods of contrasting reveal similar phenomenon. Viral particles of spherical regular shape with a size of 17–21 nm were found (Fig. 4d), which is much smaller than the typical sizes of native viral particles (contrasted with uranyl acetate). Fig. 4d shows a viral particle with a size of 19 nm. Also, deformed viral particles with significantly increased sizes (30–38 nm) were found (Fig. 4e, 4f). Fig. 4e shows micrograph made with uranyl acetate as contrasting agent, and Fig. 4f shows micrograph made with phosphotungstic acid as contrasting agent. Both micrographs show similar phenomenon.

Interaction With CeO₂ NPs

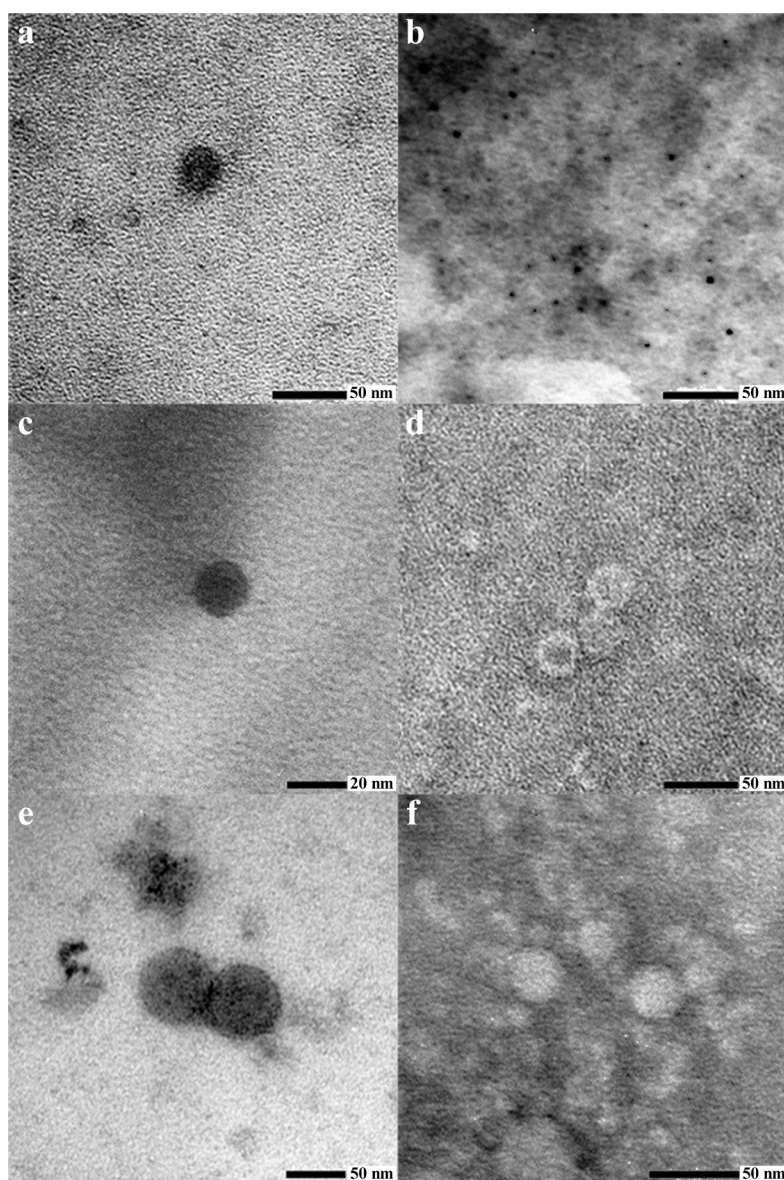
Similar results were obtained during the interaction of viral particles with CeO₂ NPs (Fig. 5). CeO₂ NPs were also showed the ability to adsorb on the surface of viral particles, and changed their morphology. As in the case of Ce NPs, the interaction of CeO₂ NPs with the viral particles of the studied strain can be roughly divided into three types: adsorption of NPs on the surface of viral particle without significant morphological changes (Fig. 5a, 5d, 5e, 5f); a significant decrease in the size of viral particles (19–22 nm) with maintenance of typical morphology (Fig. 5b); and a significant increase in the size of viral particles (up to 54 nm), accompanied by a significant deformation (Fig. 5c). Destroyed

virions were also found using phosphotungstic acid as contrasting agent (Fig. 5f).

The diameters of the viral particles in Fig. 5a, Fig. 5b and Fig. 5c are 28, 22, and 51 nm, respectively (Fig. 5), and in Figs. 5d, 5e and 5f – 28–29 nm, respectively. In Fig. 5a, and Fig. 5b, viral particles have sizes typical of teschoviruses, although in Fig. 5b the diameter of the viral particles approaches the lower boundary of typical values known from the literature (Cano-Gómez *et al.*, 2017; Zhang *et al.*, 2010; Knowles, 2006; Yamada *et al.*, 2014; <https://viralzone.expasy.org/659>), and the typical values established for the Dniprovskiy-34 strain are much smaller according to the results of the study. In Fig. 5c, the viral particle has significantly larger size than typical both according to the literature (Cano-Gómez *et al.*, 2017; Zhang *et al.*, 2010; Knowles, 2006; Yamada *et al.*, 2014; <https://viralzone.expasy.org/659>), and according to the results of the study. It is noteworthy that CeO₂ NPs lead to a more significant increase in the diameter of the viral particles, and severe deformation compared to Ce NPs. Thus, it was found that Ce NPs can interact with viral particles, which can significantly affect the infectious activity of the virus.

Determination of Antigenic Properties of the Virus Under the Influence of NPs

A purified, and concentrated virus suspension of TV-A strain Dniprovskiy-34 was used for hyperimmunization of rabbits. The virus titer was 7.5 lg TCD₅₀/cm³. Immunization of animals was carried out according to the plot presented in Tab. I. Blood sampling was carried out on the 7th day after the last injection of antigen. In the virus neutralization reaction, a titer of virus-neutralizing antibodies in blood serum was established (Tab. VII). It was found that under the introduction of antigen with NPs, neutralizing antibodies are formed, but in lower titers. A slight decrease in the titers of neutralizing antibodies was observed under the influence of CeO₂, and Ce NPs.



3: Transmission electron micrograph of the samples of TV-A strain Dniprovskiy-34, which were: purified with chloroform, and concentrated with ammonium sulfate (contrasted with uranyl acetate) (a); purified with ultracentrifuge with 15% sucrose gradient (contrasted with uranyl acetate) (b); purified with ultracentrifuge, fraction with 30% sucrose content contrasted with uranyl acetate (c); purified with ultracentrifuge, fraction with 30% sucrose content contrasted with phosphotungstic acid (d); the sample, which contains 45% of sucrose contrasted with uranyl acetate (e); and the sample, which contains 45% of sucrose contrasted with uranyl acetate contrasted with phosphotungstic acid (f)

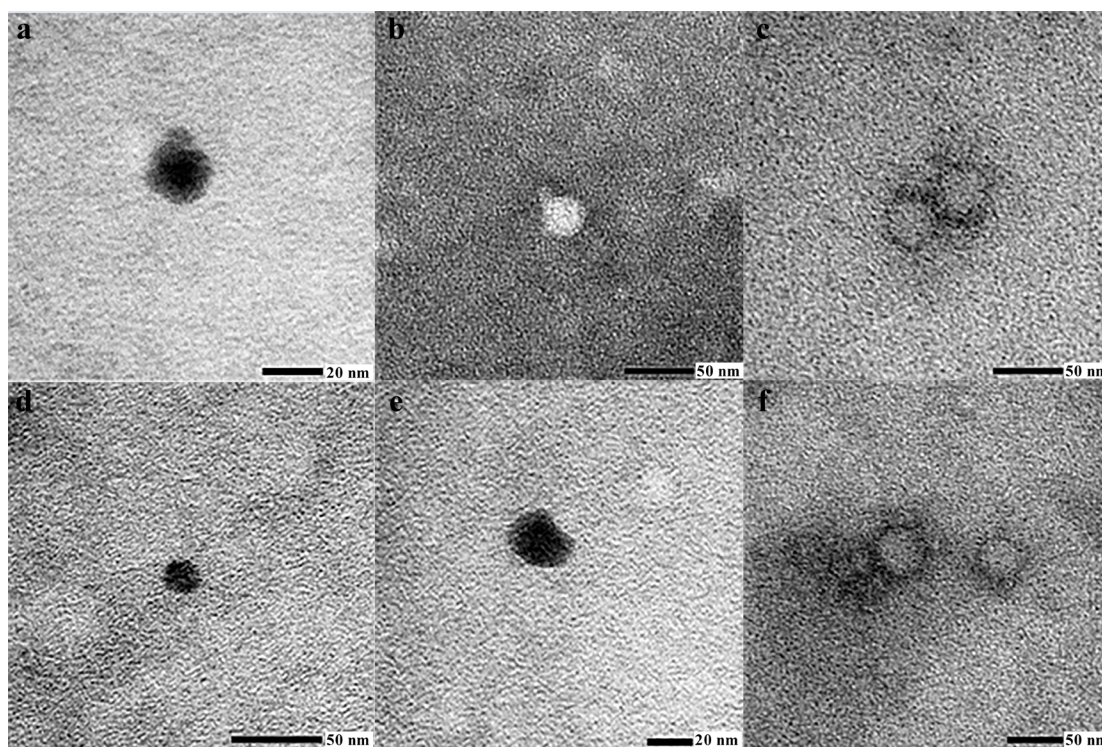
Thus, it was found that under the influence of CeO_2 , and Ce NPs, viruses retain their antigenic properties. The introduction of NPs into the antigen caused a decrease in the titer of neutralizing antibodies, however, they have remained at a high level.

DISCUSSION

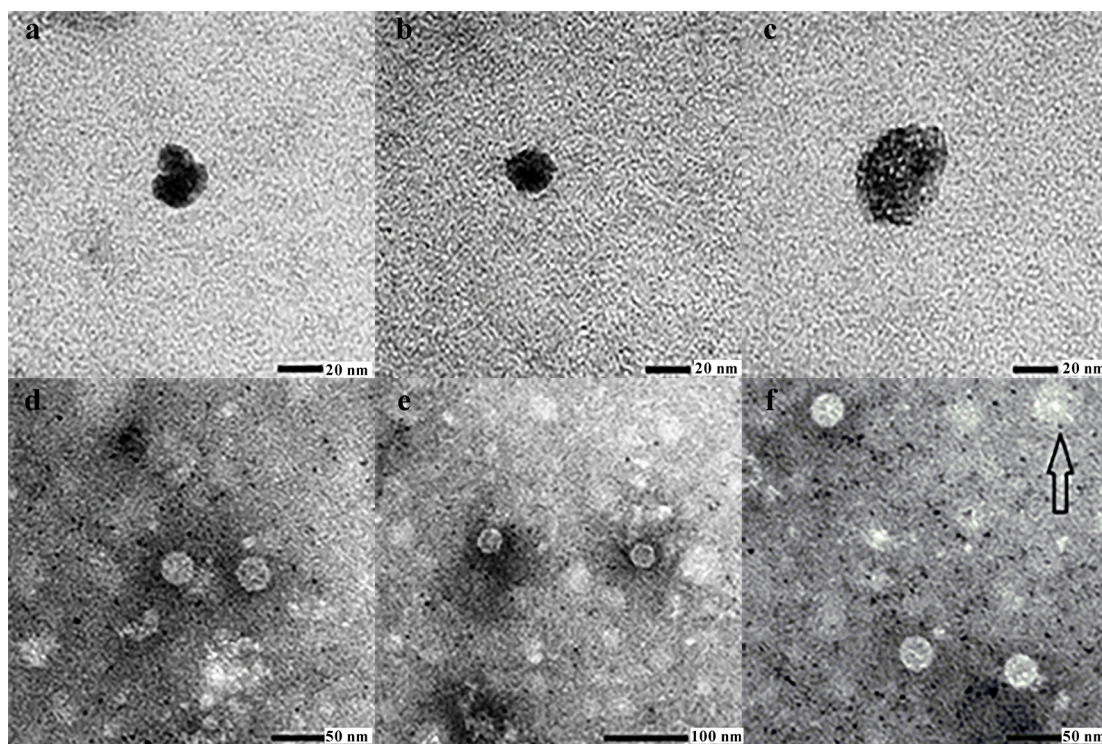
Morphology and Structure of Ce NPs

The amorphous structure of Ce NPs (which should be crystalline) can be due to the presence of

citrate anions, which can form a layer around NPs, or due to core/shell structure of NPs; an electron diffraction pattern was recorded (Fig. 1, b). Despite the high chemical activity of metallic Ce^0 , the presence of Ce NPs in the composition cannot be excluded. In particular, Ce^0 can form cores of NPs with a core/shell structure, the shell of which can be formed by more chemically inert CeO_2 , and $\text{Ce}(\text{OH})_3$. Spontaneous formation of core/shell structures has already been established for NPs of some metals (Johnston-Peck *et al.*, 2009).



4: Transmission electron micrographs of viral particles under the influence of Ce NPs: a – a viral particle with adsorbed Ce NPs (contrasted with uranyl acetate), b, c – a viral particle with adsorbed Ce NPs (contrasted with phosphotungstic acid), d – a significantly smaller viral particle (contrasted with uranyl acetate), e – a deformed viral particle with significantly increased sizes (contrasted with uranyl acetate), f – a deformed viral particle with significantly increased sizes (contrasted with phosphotungstic acid)



5: Transmission electron micrographs of viral particles under the influence of CeO_2 NPs: a – a viral particle with adsorbed CeO_2 NPs (contrasted with uranyl acetate), b – a substantially smaller viral particle (contrasted with uranyl acetate), c – a deformed viral particle with significantly increased sizes (contrasted with uranyl acetate), d, e, – viral particles with adsorbed CeO_2 NPs (contrasted with phosphotungstic acid), f – viral particles with adsorbed CeO_2 NPs, and destroyed viral particles (contrasted with phosphotungstic acid). Destroyed virions are pointed with an arrow

VII: The titer of virus-neutralizing antibodies in the hyperimmune serum of rabbits obtained for TV-A strain Dniprovskiy-34

No.	Antigen with NPs	Concentration of NPs, $\mu\text{g}/\text{cm}^3$	Titer, dilution of blood serum
1	CeO ₂	1	1:2048
2	Ce	0.1	1:2048
3	Control, antigen with NPs		1:4096

Morphology and Structure of CeO₂ NPs

An analysis of the obtained photographs indicates the presence of NPs with different electron densities in the samples (Fig. 2, a, b), which can indicate both the difference in the volume of the nanoparticles, and their spatial position in the agglomerate, as well as differences in the chemical composition. In particular, it is known that cerium in the composition of NPs exhibits a mixed valence: Ce³⁺, and Ce⁴⁺, and the fraction of ions with different valence states can differ by 27%, depending on the size of the NPs (Deshpande *et al.*, 2005), and change under the influence of other factors (Zhang *et al.*, 2015; Pulido-Reyes *et al.*, 2015).

The electron diffraction pattern does not indicate that the NPs in the studied samples have crystal structure (Fig. 2, c). The presence of a diffuse halo in the electron diffraction pattern is characteristic of substances with an amorphous structure (Suryanarayana, 1984; Sarac *et al.*, 2018), which does not exclude the presence of a small amount of nanocrystals in the agglomerates (Fig. 2c).

The Effect of Ce and CeO₂ NPs on Eukaryotic Cells as an Important Factor in Determining Their Potential for Use as Antiviral Agents

CeO₂ and Ce NPs attract the attention of researchers all over the world as promising antiviral, antibacterial and antifungal agents (Babenko *et al.*, 2012; Oksamytnyi *et al.*, 2014; Shydlovska *et al.*, 2018; Munusamy *et al.*, 2014; Alpaslan *et al.*, 2017; Gopinath *et al.*, 2015; Krishnamoorthy *et al.*, 2014). One of the reasons of such a high interest is their low cytotoxicity. Despite some studies, which report high toxicity of CeO₂ NPs (Krishnamoorthy *et al.*, 2014; Lin *et al.*, 2006), the majority of data indicate that CeO₂ NPs have low cytotoxicity (Chen *et al.*, 2006; Xia *et al.*, 2008; Zholobak *et al.*, 2011; Derevianko *et al.*, 2019) and even can have beneficial properties such as antioxidant (Chen *et al.*, 2006; Xia *et al.*, 2008) and cytoprotective (Xia *et al.*, 2008; Zholobak *et al.*, 2011) properties.

Thus, low cytotoxicity of CeO₂ NPs and their beneficial properties such as antioxidant and cytoprotective activity make them promising candidates for the developments of antiviral drugs and disinfectants.

Influence of Ce and CeO₂ NPs on Virus Titer Under Normal Conditions and Under the Action of Chemical and Physical Factors

NPs, which caused a decrease in virus titer by at least 2.0lg₁₀, inhibited virus reproduction in

a single cycle experiment by 1.25–2.0lg₁₀, and had TI 4, and more, at concentrations not higher than the TLVs for animals, and humans, were considered to express prominent activity. Such NPs are highly active, and promising for further studies in animals (Guideline of the German Association for the Control of Viral Diseases (DVV) e. V. and the Robert Koch Institute (RKI) for testing chemical disinfectants for effectiveness against viruses in human medicine. Version of 1 December, 2014; Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity in the medical area – Test method and requirements (Phase 2/Step 1); Chemical disinfectants and antiseptics – Application of European Standards for chemical disinfectants and antiseptics).

Thus, CeO₂ and Ce NPs, which, caused a decrease in virus titer by 1.46–2.0lg₁₀ TCD₅₀/cm³ at the concentration equivalent to the TLV at all stages of virus reproduction are promising for the development of antiviral drugs based on them. However, TI 2 is low. In our opinion, there is a need to improve the architecture of NPs in order to increase their biological activity (Deshpande *et al.*, 2005; Zhang *et al.*, 2001; Dunnick *et al.*, 2015; Pulido-Reyes *et al.*, 2015; Alpaslan *et al.*, 2017).

Influence of Ce and CeO₂ NPs on Viral Particles Morphology and Its Possible Mechanisms

Both Ce, and CeO₂ NPs showed the ability to adsorb on the surface of viral particles, and significantly affect their morphology, changing their size, and shape. 3 types of atypical viral, and virus-like particles, the formation of which is undoubtedly a consequence of the interaction of typical viral particles with CeO₂, and Ce NPs, were found in the studied samples:

1. Stable complexes formed by spherical viral particles with icosahedral type of symmetry, and typical dimensions, on the surface of which the NPs are attached;
2. Spherical viral particles of icosahedral type of symmetry with the size of 17–22 nm, which is significantly smaller than the sizes of native viral particles;
3. Significantly deformed viral particles up to 37–51 nm in size, which by the nature of contrast can appear to be destroyed viral particles, or virus-like particles formed by structural elements of destroyed capsids.

Therefore, the effect of Ce, and CeO₂ NPs on the viral particles of the studied strain can be divided into three conditional types:

1. Adsorption of NPs on the surface of the viral particles without significant changes in morphology;
2. Significant reduction in the diameter of the viral particles while maintaining the typical shape;
3. Significant increase in the size of viral particles in combination with strong deformation, or complete destruction of viral particles with the subsequent defective self-assembly of capsomeres in large virus-like particles of the irregular shape.

These phenomena are consistent with the literature data (Lozovski *et al.*, 2011; Lozovski *et al.*, 2012), but we have obtained confirmations of these phenomena by electron microscopy, and using TV-A as a model object for the first time. These phenomena require further research.

Influence of Ce NPs

Under the influence of Ce NPs, the diameters of the viral particles for the three mentioned types of interaction were 25, 19, and 37 nm, respectively (Fig. 4). Various sources give typical diameters of TV-A particles in the range 22–30 nm (Cano-Gómez *et al.*, 2017; <https://viralzone.expasy.org/659>; Zhang *et al.*, 2010; Knowles, 2006; Yamada *et al.*, 2014). Therefore, according to the literature, the viral particles diameter of 23.23 nm is small, but does not violate the idea of the typical size of viral particles of teschoviruses. Instead, an increase in diameter to 37 nm is highly atypical, and may indicate changes in the physicochemical properties of the viral particle. There are no reports of teschovirus viral particles of such large size in the available scientific, and patent database.

Influence of CeO₂ NPs

We showed that CeO₂, and Ce NPs were capable of adsorption on the surface of viral particles, which could lead to changes in their morphology (Fig. 5). In the available scientific, and patent database, we have not found any reports of changes in the shape of viral particles of non-enveloped viruses under the action of metals, and metal oxides NPs. It is obvious that our unique data on changes in the morphology of viral particles under the action of Ce, and CeO₂ NPs is due to the unique properties of Ce, and CeO₂ as chemicals, and, especially, their NPs, because nano-scaled chemicals acquire new properties. CeO₂ is a semiconductor with unusual properties. CeO₂ behaves as an *n-type* semiconductor (Khan and Akhtar, 2018; Nakagawa *et al.*, 2016), which may explain the exceptionally high activity of CeO₂ NPs. *N-type* semiconductors differ in that the vast majority of charge carriers in them are negatively charged electrons, while positively charged holes are less. This gives them an excessive negative charge. The large number

of free electrons causes the generation by such semiconductors a much larger amount of ROS in the aqueous medium, compared with *p-type* semiconductors, and intrinsic semiconductors. This is especially important for the biological activity of semiconductor NPs, because the generation of ROS is the main mechanism that will determine the antiviral, antibacterial, and antifungal properties of NPs of most metals, and metal oxides. The level of antimicrobial activity of NPs is the greater the more ROS is generated on their surface (Alpaslan *et al.*, 2017; Gopinath *et al.*, 2015).

CeO₂ is a powerful catalyst that catalyzes a wide range of chemical reactions, including hydrogen conversion. CeO₂ catalyzes the methanation of CO₂, and the catalytic oxidation of hydrocarbons such as toluene. CeO₂ has a high internal hydrophobicity (Peng *et al.*, 2018; Montini *et al.*, 2016). This provides high resistance against reducing catalytic activity in aqueous medium, and high ability of CeO₂ to adsorb organic compounds (Paier *et al.*, 2013).

All these properties determine the unique biological activity of CeO₂ NPs.

To date, many mechanisms of interaction of metals, and metal oxides NPs with viral particles have been described, and proposed. According to some of the proposed mechanisms, NPs affect the geometric characteristics of viral particles (Lozovski *et al.*, 2011; Lozovski *et al.*, 2012), which may explain our results.

Thus, viral particles, and NPs are able to form stable configurations. The formation of such configurations "virus-nanoparticle" changes the geometric characteristics of viral particles, which reduces their virulence (Lozovski *et al.*, 2011). The geometry of viral particles is very important factor that determines the ability of viruses to penetrate cell membranes. Due to its stability, the geometry of the "virus-nanoparticle" system differs from the geometry of the free viral particle, which reduces the efficiency of interaction of the capsid with the membrane of the sensitive cell (Lozovski *et al.*, 2012).

The effect of local fields of NPs on the surface receptors of the viral capsid can lead to modification of the molecular groups of receptors, including their complete destruction at high strength of the local field of NPs. As a result, the viral particles may completely lose their infectivity. If there are already infected cells in the cell culture, then, in the case of the presence of NPs in the culture, after leaving the infected cells, the viral particles lose infectivity due to this mechanism (Lozovski *et al.*, 2012).

If, despite changes in the geometric characteristics of the system, the "virus-nanoparticle" manages to get into the cell, then after undressing the virus, the presence of NPs in the cell can interfere with the infectious process, and block the production of new viral particles (Lozovski *et al.*, 2012).

Penetration of the NPs to an already infected cell interferes with the reproduction of the virus,

causing the destruction of viral DNA, and RNA (Lozovski *et al.*, 2012).

The binding energy of the NPs to the viral particles is the greater the smaller the size of the NPs. It is the largest for NPs smaller than 10 nm (Lozovski *et al.*, 2012).

Local fields of NPs affect viral genome replication, and capsid assembly, but *in vivo* this effect can be reduced by external fields (Lozovski *et al.*, 2012).

The reduction in size of viral particles can be explained by the destruction of the surface proteins possibly by ROS generated by CeO₂ and Ce NPs.

Enlarged, and deformed viral particles with a size of 37–51 nm may have formed as a result of the destruction of viral capsids.

Influence of CeO₂ and Ce NPs on Antigenic Properties

The introduction of CeO₂ and Ce NPs into the antigen caused a decrease in the titer of virus-neutralizing antibodies; however, the concentration remained at a high level.

Thus, the cytotoxic effect of CeO₂ and Ce NPs in the culture of SKECL cell line, and their toxicity on white mice have been studied. The antiviral activity of nanoparticles at different stages of virus reproduction has been established. The biological, and physicochemical properties of the virus under the influence of NPs have been studied. The mechanism of the interaction between CeO₂ and Ce NPs with viruses has been elicited via transmission electron microscopy. The antigenic properties of the virus under the influence of NPs have been studied. The results obtained in the research can be used in the development of disinfectants, and antiviral drugs.

CONCLUSION

It has been found that the TLVs for cell culture of SKECL cell line are 1.0 µg/cm³ for CeO₂ NPs, and 0.1 µg/cm³ for Ce NPs. The studied NPs are not toxic to white mice at a concentration of 2000 mg/kg. Reproduction of Dniprovskiy-34 strain of *Teschovirus A* species of *Porcine teschovirus-1* serotype under the influence of CeO₂ and Ce NPs according to prophylactic, therapeutic, and virucidal plots has been studied in experiments using SKECL cell culture. It was found that according to the prophylactic plot CeO₂ and Ce NPs have caused a decrease in the virus titer by 2.0 lg TCD₅₀/cm³, according to the treatment plot - by 1.5 lg TCD₅₀/cm³. CeO₂ and Ce NPs have reduced the virus titer by 1.46, and 2.0 lg TCD₅₀/cm³, respectively, according to virucidal plot.

It has been found that CeO₂ and Ce NPs cause a slight decrease in the infectious titer of the virus by 0.23 lg TCD₅₀/cm³, under the action of solvents, and proteolytic enzyme trypsin. In heating tests, and at different pH values CeO₂ and Ce NPs have not shown antiviral activity.

It has been established that CeO₂ and Ce NPs can be adsorbed on the surface of viral particles, and change their morphology.

Viruses maintain their antigenic properties under the influence of NPs. The introduction of NPs into the antigen caused a decrease in the titer of virus-neutralizing antibodies; however, it remained at a high level. These results will be used in the future in the development of disinfectants, and antiviral drugs.

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