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Structure-sensitive properties and biomedical applications of nanodispersed cerium dioxide

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Abstract. The results of modern studies of physicochemical properties and biological activities of nanocrystalline cerium dioxide are generalized. The key methods for its preparation are considered and the prospects for its application in biological research and in medicine are analyzed. The bibliography includes 119 references.

I. Introduction

The use of cerium compounds as drug components has a long history: the first publications on this subject appeared in the 1850s. In the vast majority of cases, water-soluble cerium(III) salts (nitrate, chloride, sulfate or acetate) and some insoluble compounds (for example, cerium oxalate or stearate), *e.g.*, as colloid solutions are used as the active components.[1]

The mechanism of action of cerium(III) on the organism is not entirely clear. It was noted[1] that the biological activities of cerium salts can in some cases be due to the similarity of the Ce^{3+} and Ca^{2+} ionic radii; therefore, cerium ions can partially replace calcium ions in a number of biomolecules. This, in particular, can account for the anticoagulating action of cerium and other lanthanide compounds. In addition, cerium(III) salts are well known to be used as antiemetic, bacteriostatic, bactericidal, immunomodulating and antitumour agents.[1,2] For example, flammacerium, a 1% solution of silver sulfadiazine and cerium nitrate, is an efficient local agent for treating burn wound infections and infectious complications of the burn disease. Apart from direct antiseptic action, cerium(III) salts help to prevent postburn sepsis and systemic inflammatory response by binding the burn toxins.

Currently, the undeservedly forgotten antitumour potential of cerium-based agents is studied anew; in particular, Ji *et al.*[2] demonstrated the ability of cerium chloride to inhibit proliferation of gastric cancer cells (PAMC82 line) and leukemia cells (K562 line). Nevertheless, the mechanisms that underlie the activity of Ce^{3+} compounds are still to be explained.[1]

The role of cerium compounds in agriculture, first of all in crop growing and cattle breeding, increases. For example, Fashui[3] studied the effect of cerium nitrate pretreatment on the rice germination: the considerable positive effect was attributed to increasing the intensity of seed breathing, increasing the superoxide dismutase (SOD), catalase and peroxidase activities, and decreasing the content of the superoxide ion and malonaldehyde. In the early 1990s, lanthanide complexes were studied as regards their use in biology and agriculture at the Tver State University; large experience in using lanthanides for cattle breeding was accumulated in China. In the late 1990s, researchers from the Ludwig Maximilian University of Munich addressed the problem of using lanthanides as fodder additives. The animals that received a typical diet with addition of lanthanide salts demonstrated a considerable increase in the daily weight gain (up to 19%) and the efficiency of food assimilation increased by 11%.[4,5] Lanthanides are apparently non-toxic for animals because this additional nutrition did not affect the blood serum thyroxine, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total cholesterol, total protein, albumin, glucose or Ca, P, Na, K and Cl levels. The accumulation of cerium and lanthanum in muscles, liver and kidneys three weeks after the 'lanthanide diet' was insignificant. The possibility of using lanthanides as safe and inexpensive alternative fodder supplements in pig breeding was demonstrated.[6,7] Due to toughening of WHO requirements on the use of antibiotics in additional nutrition of domestic animals, the use of cerium (and some other lanthanide) compounds appears fairly promising. In 2003, a lanthanum – cerium agent was registered in Switzerland (under the trade name Lancer®),

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its recommended doses are 150 to 300 mg of lanthanide per kg of the fodder.

Data on the biological activities of cerium dioxide are much more fragmentary; until recently, this compound has hardly received attention because cerium dioxide is insoluble in water or biological fluids. Therefore, of special interest are the prospects for biomedical applications of nanocrystalline cerium dioxide reported in the last five years.

It is noteworthy that published data on the structure and properties of nanodispersed CeO_{2-x} are often contradictory; moreover, many properties of nanocrystalline cerium dioxide have scarcely been studied till now. In this review, we consider the key size effects inherent in CeO_{2-x}, analyze the most important methods for the study of nanocrystalline cerium dioxide, generalize the available data on its biological activity and describe the most effective methods for its preparation.

II. Structure and properties of nanocrystalline cerium dioxide

As the particle size decreases, the unit cell parameters of many substances (*e.g.*, gold [8]) decrease due to the increase in the surface tension. Nevertheless, recently it has been shown that for some oxide compounds (including CeO_{2-x}), the opposite trend occurs: the unit cell parameter increases with a decrease in the particle size. This was discovered for the first time in a study of almost monodisperse cerium dioxide particles with sizes of 6.7, 3.8 and 2.1 nm, which were isolated from highly acidic sols stabilized by anionic surfactants.[9,10] The particle size and lattice parameters were determined by transmission electron microscopy and electron diffraction. Figure 1 presents the dependence of the unit cell parameter on the CeO_{2-x} particle size obtained by Tsunekawa *et al.*[9] In the authors' opinion, the observed pattern of variation of the unit cell parameter is caused by gradual decrease in the cerium effective oxidation number as a result of removal of some oxygen atoms located on the particle surface from their crystallographic sites to give oxygen vacancies. According to calculations,[9–11] the critical particle size corresponding to complete $Ce^{4+} \rightarrow Ce^{3+}$ transition is 1.9 nm (Table 1). The proposed model of partial reduction of cerium includes protonation of surface oxygen atoms followed by elimination of water to give oxygen vacancies.

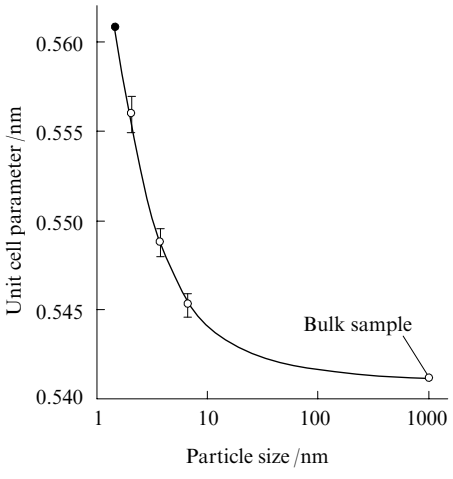


Figure 1. Unit cell parameter *vs.* CeO_{2-x} particle size for nanocrystalline and coarsely crystalline samples.[9]
The dark circle corresponds to the model case with Ce^{3+} content of 100%.

Table 1. Size and composition of CeO_{2-x} nanoparticles.[9]

Particle size /nm	Fraction of cerium atoms in the surface layer (%) (calculated)	Fraction of Ce^{3+} (%) (experiment)	Nominal particle composition
7.0	27	21	$CeO_{1.894}$
4.6	43	38	$CeO_{1.810}$
2.6	75	74	$CeO_{1.630}$
1.9	100		

The results of computer simulation [12] indicate that for substances with predominantly ionic type of bond (in particular, for oxides), the change in the unit cell parameter on passing to the nanostate is related to the change in the formal oxidation state of atoms. The decrease in the formal oxidation number inevitably results in increase in the unit cell parameter caused by attenuation of electrostatic forces, whereas the increase in the formal oxidation number may cause either decrease or increase in the unit cell parameter depending on the compound structure.

More recently,[13–15] additional experimental evidence for increasing of the unit cell parameter of cerium dioxide following a decrease in the particle size has been obtained. In particular, analysis of CeO_{2-x} nanoparticles (3–20 nm) prepared from the gas phase was reported.[13] The unit cell parameters varied in the range of 0.541–0.561 nm depending on the particle size, this range being somewhat wider than that reported in earlier studies.[9–12] It was assumed [13] that this discrepancy is due to more non-equilibrium conditions of preparation of CeO_{2-x} nanoparticles. It was shown by electron energy loss spectroscopy that the oxygen non-stoichiometry of nanoparticles increases from the middle to the surface. Thus, cerium dioxide nanoparticles are actually core–shell nanostructures; in relatively large particles, the core has a composition close to stoichiometric cerium dioxide and the surface is close to Ce_2O_3. The measurements [13] demonstrated that the thickness of the surface layer is different for CeO_{2-x} particles of different size (Fig. 2) and that the fraction of Ce_2O_3 sharply increases with a decrease in the particle size. The effective oxidation

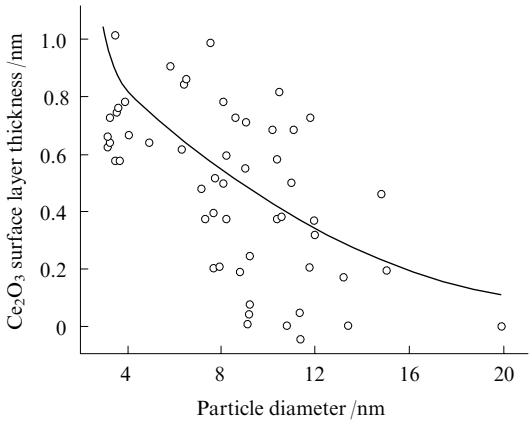


Figure 2. Thickness of the Ce_2O_3 surface layer for CeO_{2-x} particles of different size (with the assumption that Ce^{3+} ions are located only near the surface).[13]

number of cerium becomes +3 for particles 3 nm in diameter (or 1.9 nm according to other estimates[9]).

According to Zhang *et al.*,[14] for CeO_{2-x} particles with a size of 6.1–80 nm obtained by homogeneous precipitation from cerium(III) nitrate in the presence of hexamethylenetetramine, the unit cell parameter varies from 0.543 to 0.541 nm (Fig. 3). Note that both the proper values and their variation range are much smaller than those reported in other publications.[9,13] Similar data (unit cell parameter in the range of 0.550–0.541 nm for 5–30 nm particles) were found relying on the Rietveld refinement of structural parameters in our study of several series of nanocrystalline cerium dioxide samples prepared by fast and homogeneous precipitation.

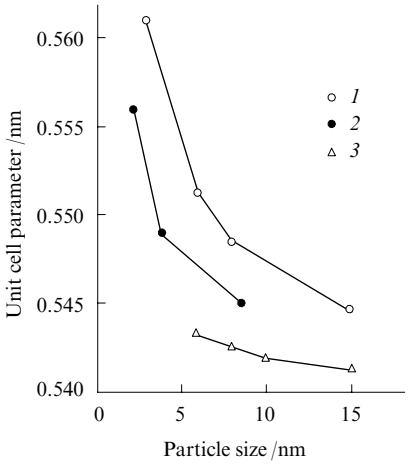


Figure 3. Unit cell parameter *vs.* CeO_{2-x} particle size from Ref. 13 (*1*), Refs 9, 16 (*2*) and Ref. 14 (*3*).[17]

The unit cell parameters for highly aggregated CeO_{2-x} particles synthesized by 'soft chemistry' methods in the presence of hydrogen peroxide follow the same trend but the range of values is 0.542–0.560 nm,[15] which is well correlated with the data obtained by Wu *et al.*[13] In the opinion of Deshpande *et al.*,[15] the increase in the unit cell parameter is due to partial reduction of Ce^{4+} ions on the particle surface with hydrogen peroxide. The following data on Ce^{3+} concentration in CeO_{2-x} nanoparticles of different size were obtained:[15]

Particle size /nm	3	6	30
Ce^{3+} content (%)	44	29	17

Figure 3 presents the dependences of CeO_{2-x} crystal lattice parameter on the particle size obtained in various studies. This clearly illustrates the above-mentioned discrepancy between published data. This discrepancy was attributed[16,17] to different CeO_{2-x} nanoparticle shape: greater slopes of the dependence were observed for spherical or ellipsoid particles,[9,13] whereas smoother dependences are typical of octahedral particles.[14] Since the oxygen non-stoichiometry of cerium dioxide is correlated with its catalytic activity, the researchers cited stated that CeO_{2-x} nanopowders composed of particles without clear-cut faceting are more desirable for catalytic applications.

However, the body of experimental data accumulated to date is relatively small, the actual reasons for differences in the lattice parameter of CeO_{2-x} nanoparticles synthesized by different methods are still unknown. In particular, the oxygen non-stoichiometry of the surface layers of CeO_{2-x} nanoparticles can depend considerably on the nature of the molecules and ions adsorbed on their surface. Furthermore, an additional increase in the oxygen non-stoichiometry can be due to the synthesis of cerium dioxide nanoparticles under highly non-equilibrium conditions. This result can be caused, for example, by high-temperature synthesis of CeO_{2-x} in an atmosphere with low partial content of oxygen followed by fast quenching.[18]

The hypothesized[9] dependence of the oxygen nonstoichiometry on the particle size of nanocrystalline cerium dioxide is confirmed by analysis of Raman spectroscopy data. In was demonstrated[19,20] that a decrease in the CeO_{2-x} particle size induces systematic changes in the Raman spectra; in particular, the position and the width of the peak corresponding to the triply degenerate active mode F_{2g} (464 cm^{-1}) change. The experimental dependence of the half-width of this line on the reciprocal particle size is shown in Fig. 4.

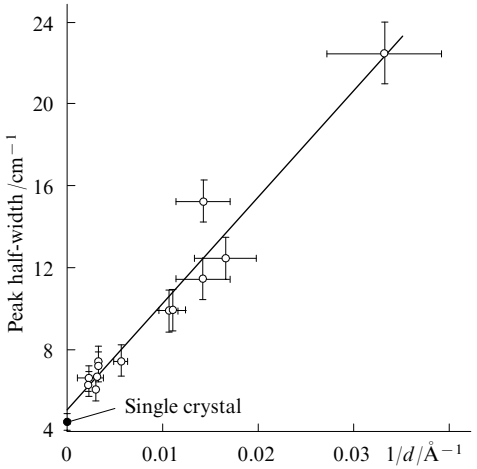


Figure 4. Half-width of the Raman peak at 464 cm^{-1} *vs.* reciprocal particle size.[20]

It was noted[21,22] that the mode at 464 cm^{-1} corresponding to CeO_8 symmetric vibrations should be exceptionally sensitive to any violation of the oxygen sublattice symmetry, in particular, to changes in the oxygen stoichiometry of cerium dioxide caused by thermal treatment and/or size effects. This conclusion was indirectly confirmed by Raman spectra of the lanthanide-doped cerium dioxide samples: the observed shifts in the line positions depend first of all on the concentration of oxygen vacancies, which is in turn determined by the content of the additive.[23]

Analysis of the Raman spectra of cerium dioxide thin films with different particle size (4–300 nm) showed[21,22] that the key effects that accompany the decrease in the size of crystallites are broadening of the peak at 464 cm^{-1} and simultaneous increase in the peak asymmetry (Fig. 5) caused by the decrease in the phonon lifetime typical of nanocrystalline materials.[20] The linear dependence of the peak half-width on the reciprocal particle size was confirmed. Note that similar dependences were also obtained previously for other nanocrystalline materials (Si, Ge, GaAs, *etc.*).

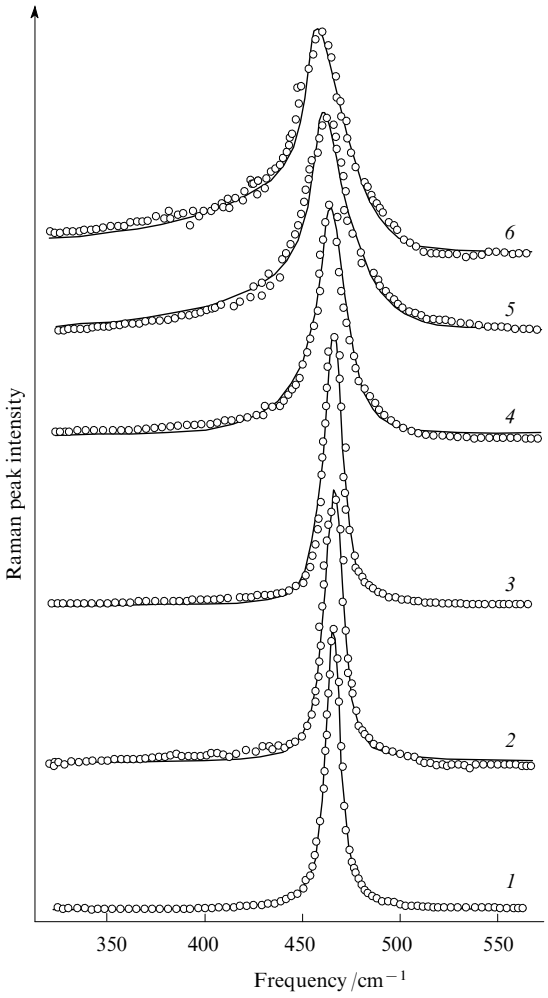


Figure 5. Raman spectra of single crystalline (*1*) and nanocrystalline (*2*–*6*) of CeO_{2-x} samples.[22]
Particle size /nm: 150 (*2*), 64 (*3*), 20 (*4*), 9 (*5*), 4 (*6*). Continuous lines are the model, the dots are experimental values.

A spatial correlation model was proposed for describing the structure of nanocrystalline cerium dioxide.[21,22] The introduced correlation length parameter corresponds to the mean size of the area in which the spatial homogeneity of the material is retained. When the concentration of oxygen vacancies is 10^{21} cm^{-3}, almost every unit cell of cerium dioxide contains defects, and the correlation length corresponds to the oxygen–oxygen distance in the cell (2.71 Å). For single crystals with very low degree of non-stoichiometry, the correlation length tends to infinity. This model can describe satisfactorily the peak shape in the Raman spectra of CeO_{2-x}; moreover, the concentration of oxygen vacancies (10^{17}–10^{20} cm^{-3}) in nanoparticles of different size calculated from the correlation lengths coincides well with this value found by coulometric titration and electrical conductivity measurements.

It was shown[24] that the shift of the Raman peaks to lower energies is determined by the increase in the unit cell parameter of cerium dioxide following the decrease in the particle size. In turn, an increase in the peak width is caused by the presence of inhomogeneous lattice stress. High-temperature annealing of CeO_{2-x} nanoparticles induces irreversible change in the Raman peak position (Fig. 6)

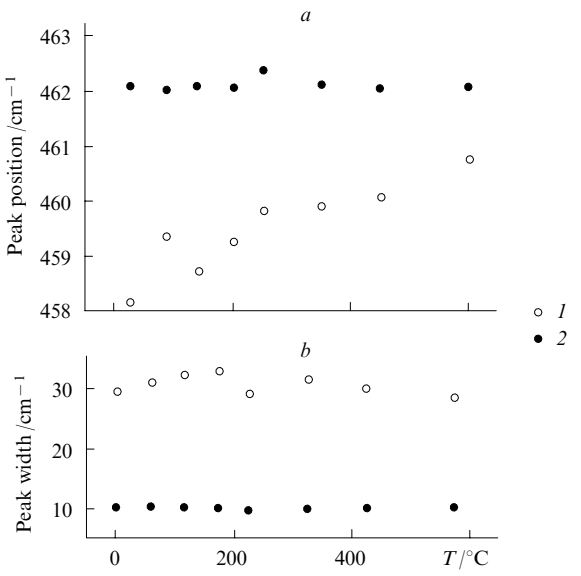


Figure 6. Changes in the Raman peak position (*a*) and width (*b*) after high-temperature annealing for nanocrystalline CeO_{2-x} samples with particle size of 6.1 (*1*) and 25 nm (*2*).[24]

caused, in the authors' opinion,[24] by stress relaxation and/or oxidation of non-stoihiometric cerium dioxide.

A detailed comparison of the data from powder X-ray diffraction, Raman spectroscopy and low-temperature nitrogen adsorption for a series of CeO_{2-x} samples synthesized by the sol–gel technique was carried out.[25] The factors in the equation relating the Raman peak width (Γ /cm^{-1}) to the reciprocal crystallite size ($1/d$) were found to depend on the chemical prehistory of the material (d, nm):

$$\Gamma = 5 + \frac{51.8}{d} \text{ (see Ref. 20)},$$

$$\Gamma = 10 + \frac{124.7}{d} \text{ (see Ref. 21)},$$

$$\Gamma = 5.48 + \frac{98.4}{d} \text{ (see Ref. 19)}.$$

According to X-ray photoelectron spectroscopy (XPS) data,[26–28] apart from the Ce^{4+} ions (electronic configuration $4f^0$), cerium dioxide can actually contain cerium ions in an intermediate (lower) oxidation state ($4f^n$, $n \approx 0.5$). However, it was found experimentally[29,30] that partial reduction of cerium can take place directly in the spectrometer work space during the measurements under X-ray irradiation of CeO_{2-x} samples in high vacuum (Table 2), which must be taken into account in interpretation of the XPS data. It was assumed[29,30] that this effect may be a result of local overheating of samples during the measurements. The possibility of partial reduction of cerium dioxide during recording of the XPS spectra is confirmed in another publication.[31] The authors believe that reduction may take place only in the case where the surface of the analyzed samples is hydroxylated. Note that this hypothesis was disputed by Paparazzo and Ingo,[32] who argue that the cited measurements[31] were performed improperly.

Table 2. Binding energy for CeO_2 and Ce_2O_3 samples exposed to X-rays (Al K_α, $h\nu$ = 1486.6 eV) in high vacuum (5×10^{-10} mm Hg) during different time intervals.[29]

Sample	Irradiation time /min	Binding energy /eV	
		Ce $3d_{5/2}$	Ce $3d_{3/2}$
CeO_2	15	882.1	900.6
		888.4	907.1
		898.1	916.5
	45	882.1	900.6
		888.5	907.2
		898.2	916.5
		885.2	903.8
	300	882.1	900.7
		888.6	907.1
		898.2	916.5
		885.3	903.9
Ce_2O_3	15	880.9	899.7
		885.3	903.8

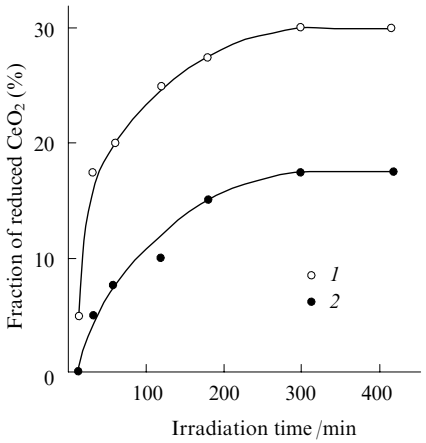


Figure 7. Reduction of CeO_{2-x} samples during recording of X-ray photoelectron spectra.[37]
The samples were synthesized at 200 (*1*) and 750 °C (*2*).

It was noted[33] that no significant reduction of cerium dioxide takes place during short-term (10 min) recording of XPS spectra at a temperature of up to 600 °C in high vacuum, whereas in a hydrogen atmosphere (10^{-2} Pa), the reduction of samples starts above 300 °C. When the hydrogen pressure was increased to 100 kPa, partial reduction of cerium dioxide was found to occur even at room temperature.[34] The reduction of cerium dioxide in a hydrogen atmosphere occurs in two steps, only one surface layer of cerium ions being reduced on heating the samples up to 650 °C.[35] Studies[36] of the oxidation processes of partially pre-reduced cerium dioxide showed that Ce^{3+} ions are present on the surface of CeO_{2-x} samples even after they have been kept in an oxygen atmosphere.

It was found[37] that the dynamics of cerium dioxide reduction during recording of the X-ray photoelectron spectra can be determined by not only such factors as irradiation energy and the composition of the atmosphere in the spectrometer cavity, but also by the sample dispersity. In particular, while studying CeO_{2-x} samples with particle size of 2.2 and 35 nm, Park and Ledford[37] found that highly dispersed samples undergo more extensive reduction, the reduction rate being also much higher (Fig. 7).

Tsunekawa *et al.*[38] carried out the first XPS analysis of the oxygen non-stoichiometry of CeO_{2-x} nanoparticles of different size. Deconvolution of the XPS spectra (Fig. 8)

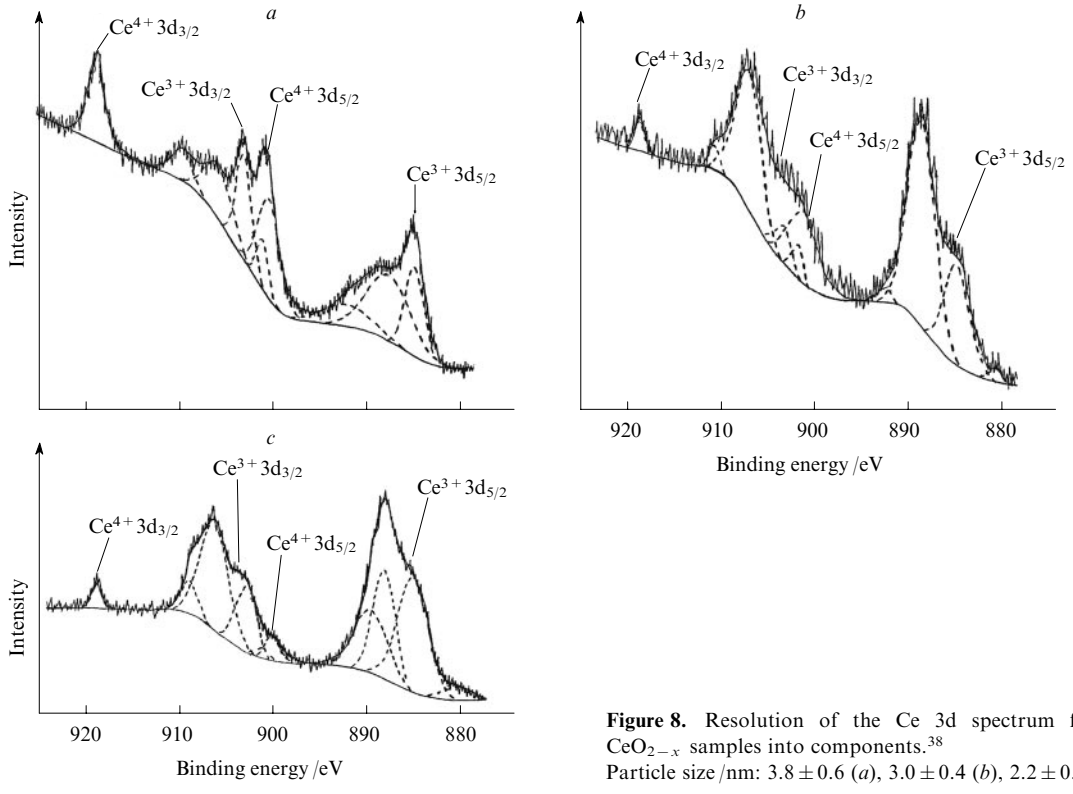


Figure 8. Resolution of the Ce 3d spectrum for monodispersed CeO_{2-x} samples into components.[38]
Particle size /nm: 3.8 ± 0.6 (*a*), 3.0 ± 0.4 (*b*), 2.2 ± 0.3 (*c*).

clearly proves the increase in the Ce^{3+} content for more highly dispersed samples. The authors noted that the obtained data are in satisfactory correlation with the oxygen non-stoichiometry data for CeO_{2-x} nanoparticles obtained by electron diffraction.

Subsequently, it was shown [39] that XPS determination of the Ce^{3+} content in nanocrystalline CeO_{2-x} samples gives considerably overestimated values, which is caused by reduction of the cerium dioxide surface layer under X-ray radiation in high vacuum even when the time of analysis is short. For overcoming this problem, it was proposed [39] to consider CeO_{2-x} nanoparticles as composed of a core, whose oxygen non-stoichiometry is largely determined by the size factor, and the shell, which is reduced to cerium(III) oxide during the measurements. The results obtained in terms of this model are summarized in Table 3.

Table 3. Characteristics of CeO_{2-x} samples of different dispersity in terms of the 'core + shell' model.[39]

Particle size /nm	Concentration of Ce^{3+} in the surface layer (%)	Surface layer thickness /Å	Concentration of Ce^{3+} in the particle bulk (%)
6	25.2	2.2	10.6
10	22.3	1.9	5.6
5000	11.6	0.9	0.0054

It should be noted that even in the framework of this approach, the Ce^{3+} concentration found by XPS is markedly higher than that determined by independent methods. It should also be taken into account that the Ce^{3+} content in the analyzed samples can depend on not only the particle size but the conditions of sample preparation. Thus it was found by XPS [40] that nanocrystalline cerium dioxide samples obtained by annealing at 250 °C have a lower degree of non-stoichiometry than similar samples annealed at 650 °C, although the particle size in the latter case (12 nm) is substantially larger than that in the former case (9 nm). Analogous results were obtained [41] by comparative analysis of micro- and nanocrystalline CeO_{2-x} samples from different sources.

The above-described changes in the oxygen non-stoichiometry upon a decrease in the nanoparticle size directly affect the electronic properties of cerium dioxide, which becomes a large-gap semiconductor. Masui *et al.*[42] carried out the first analysis of the absorption spectrum of the CeO_{2-x} colloid particles with 2.6 and 4.1 nm size synthesized in reverse micelles. It was found that a decrease in the particle size is accompanied by an increase in the band gap energy (E_g) from 2.73 to 2.87 eV for indirect transition and from 3.38 to 3.44 eV for direct transition (Fig. 9). A comparison of the results with these values found previously for CeO_2 thin films led researchers [42] to the conclusion that a change in the band gap energy is not related to quantum size effects. Nevertheless, this conclusion does not appear quite correct because the comparison was carried out with nanocrystalline films rather than bulk samples.

The E_g values for colloid solutions of cerium dioxide nanoparticles with average sizes of 3.2 ± 0.5, 2.7 ± 0.4 and 2.2 ± 0.3 nm were determined by UV spectroscopy.[43] The band gap energy for direct transition was found to increase from 3.60 to 3.90 and 4.12 eV, respectively, as the particle size decreases. The found E_g values markedly exceed those

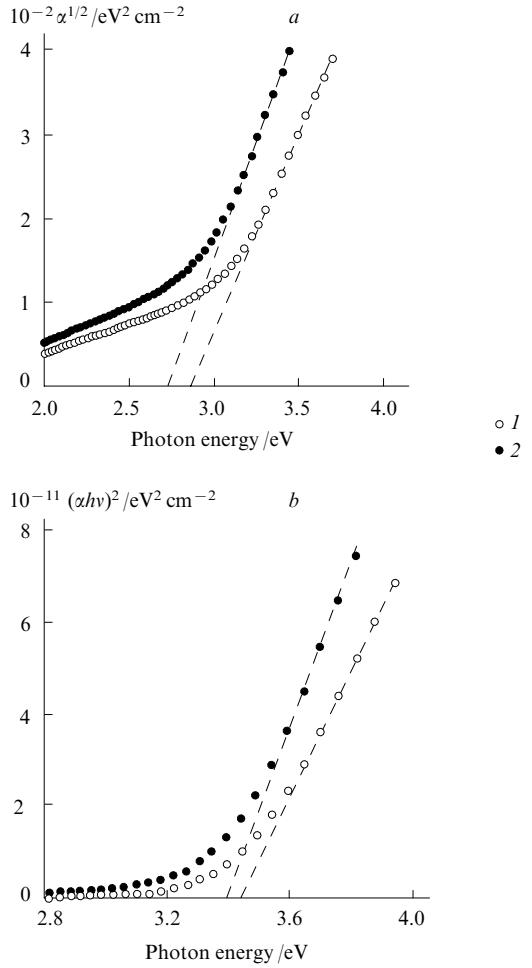


Figure 9. Determination of the band gap width for cerium dioxide nanoparticles of different size for indirect (*a*) and direct (*b*) transitions.[42]
CeO_{2-x} particle size /nm: 2.6 (*1*), 4.1 (*2*).

reported earlier.[42] A possible reason for this discrepancy is the exceptionally high error of E_g determination inherent in linearization of UV spectroscopy data.[43] According to researchers' opinion,[43] the observed change in E_g is due to the fact that a decrease in the CeO_{2-x} particle size is accompanied by an increase in their non-stoichiometry. Indeed, partial reduction of Ce^{4+} to Ce^{3+} increases the energy difference between the O 2p and Ce 4f orbitals, which correspondingly results in a hypsochromic shift of the absorption band of CeO_{2-x}.

A study of thin films of nanocrystalline cerium dioxide provided [44] additional evidence for the fact that a decrease in the particle size leads to an increase in the band gap for both direct and indirect transitions (Fig. 10). Note that this result is at variance with the results obtained by other researchers (see, for example, Ref. 45) according to which the band gap narrows down upon a decrease in the particle size. It was assumed [45] that cerium dioxide does not tend to show quantum size effects, the observed red shift of the absorption band being caused by partial reduction of the nanoparticle surface. Thus, the currently available experimental data on the effect of oxygen non-stoichiometry on the semiconductor properties of cerium dioxide are highly contradictory.

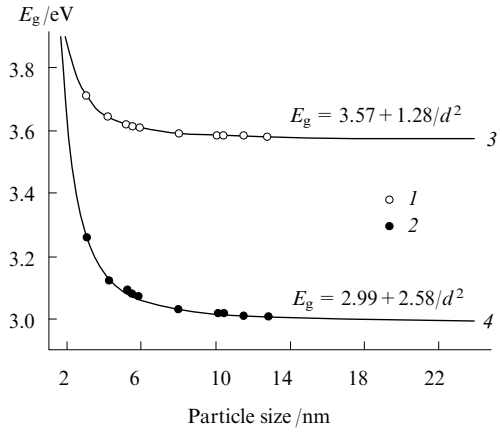


Figure 10. Band gap energy *vs.* CeO_{2-x} particle size for direct (*1*) and indirect (*2*) transitions.[44]
Lines *3* and *4* correspond to calculations within the quantum size model.

Zhang *et al.*[46] proposed a semiempirical equation that quantitatively describes the dependence of the CeO_{2-x} band gap for direct transition on the particle size

$$E_g = E_{bulk} + \frac{\hbar\pi^2}{2R^2}\left(\frac{1}{m_e} + \frac{1}{m_h}\right) - \frac{1.8e^2}{\varepsilon R},$$

where E_g is the measured band gap width of CeO_{2-x} nanoparticles, E_{bulk} is the band gap width for coarsely crystalline cerium dioxide, $\hbar$ is the Planck constant, R is the nanoparticle radius, m_e and m_h are electron and hole effective weights, respectively, and ε is the relative dielectric constant of cerium dioxide equal to 24.5. It was found that the CeO_{2-x} particle sizes in sols determined by this equation are in good agreement with the results of direct TEM measurements. The authors note that data from UV spectroscopy can be used to monitor the CeO_{2-x} particle growth during the synthesis.

The results of a study[47] confirm the dependence of the CeO_{2-x} band gap width on the nanoparticle size. Note that the E_g values found in this study[47] (3.45 and 3.68 eV for nanoparticles 3.6 and 3.3 nm in diameter) exceed the corresponding values for CeO_{2-x} particles of approximately the same size reported by Masui *et al.*[42] Analysis of the UV absorption spectra[47] suggests that the band gap width for CeO_{2-x} nanoparticles is determined by not only their size but also by the conditions of synthesis, which can affect the oxygen nonstoichiometry of the final products.

This assumption is confirmed[48] by the data for nanocrystalline CeO_{2-x} samples synthesized by the solvothermal method. It was found that the band gap for nanoparticles is generally much smaller than that for bulk CeO_{2-x} samples; however, upon a change in the nanoparticle size, the E_g remains almost invariable (Table 4). It was shown that the synthesis of CeO_{2-x} from cerium(IV) compounds results in the formation of samples with narrower band gap compared to the samples synthesized from cerium(III) salts. In other words, precipitation from solutions of cerium(IV) gives more stoichiometric cerium dioxide samples. No explanation for this fact and for the lack of E_g dependence on the particle size was reported in the study cited.[48]

Table 4. Band gap energy for CeO_{2-x} nanoparticles of different size synthesized by solvothermal method from cerium(III) nitrate and ammonium hexanitratocerate(IV).[48]

CeO_{2-x} from $Ce(NO_3)_3$			CeO_{2-x} from $(NH_4)_2[Ce(NO_3)_6]$		
particle size /nm	E_g /eV		particle size /nm	E_g /eV	
	direct transition	indirect transition		direct transition	indirect transition
2.6	3.42	2.73	2.9	3.30	2.34
3.0	3.43	2.76	3.6	3.32	2.48
3.7	3.44	2.74	4.7	3.33	2.49
4.6	3.42	2.70	6.9	3.28	2.31
6.9	3.38	2.56			

Thus, analysis of the available publications indicates that the dependence of the properties (first of all, oxygen non-stoichiometry) of nanocrystalline cerium dioxide on the chemical prehistory and the size effect requires additional integrated investigations to resolve the above noted contradictions. In turn, it will be shown below that it is the oxygen nonstoichiometry that is responsible for the unique biological activity of nanocrystalline cerium dioxide.

III. Characteristic features of the impact of nanodispersed cerium dioxide on biological systems

The prospects of using nanocrystalline cerium dioxide for the action on biological systems are determined by two key factors: high oxygen non-stoichiometry and low toxicity inherent in this material. The former factor is responsible for the activity of nanocrystalline CeO_{2-x} in biochemical redox processes especially in inactivation of reactive oxygen species (ROS) including free radicals in the living cell. The latter factor ensures relative safety of the use of CeO_{2-x} nanoparticles *in vivo*. The specific properties of CeO_{2-x} include the capability of regeneration, which implies that cerium dioxide nanoparticles that have participated in a redox process can return to the initial state (as regards the oxygen non-stoichiometry) over a relatively short period of time.

It was found[49] that the mechanism of free radical inactivation by cerium dioxide nanoparticles is similar to the action of superoxide dismutase. XPS and UV-spectroscopic studies of the reactions of cerium dioxide nanoparticles with hydrogen peroxide have shown that an increase in the $Ce^{3+}:Ce^{4+}$ ratio in a nanoparticle is directly correlated with its increasing ability to function like SOD. These results demonstrate that the size and the state of the surface of CeO_{2-x} particles are crucial in inactivating the superoxide free radicals, the presence of cerium(III) in the surface layer being the most significant. Indeed, the superoxide deactivation by superoxide dismutase is known to occur in two steps, the radical dismutation giving rise to relatively low-toxic hydrogen peroxide and oxygen; and SOD is simultaneusly regenerated. For copper-containing SOD, the processes can be represented as follows:

$$O_2^{-\bullet} + 2H^+ + (Cu^+)SOD \longrightarrow H_2O_2 + (Cu^{2+})SOD,$$

$$O_2^{-\bullet} + (Cu^{2+})SOD \longrightarrow O_2 + (Cu^+)SOD.$$

The overall process is given by

$$O_2^{-\bullet} + O_2^{-\bullet} + 2H^+ \longrightarrow H_2O_2 + O_2.$$

For CeO_{2-x} nanoparticles, the overall process is the same; it includes two steps, and the cerium effective oxidation state in CeO_{2-x} finally remains the same

$$O_2^{-\bullet} + 2H^+ + Ce^{3+} \longrightarrow H_2O_2 + Ce^{4+},$$

$$O_2^{-\bullet} + Ce^{4+} \longrightarrow O_2 + Ce^{3+}.$$

Moreover, it was stated [50] that cerium dioxide nanoparticles (unlike SOD) can inactivate also the hydroxyl radical

$$HO^{\bullet} + Ce^{3+} \longrightarrow HO^- + Ce^{4+}.$$

This fact was supported in another work [51] in which the ability of 3–5-nm cerium dioxide nanoparticles in concentrations of 1 and 1×10^{-2} mmol litre^{-1} to inactivate both the superoxide and hydroxyl radicals was ascertained by EPR. The antioxidant activity of nanoparticles increases with a decrease in their concentration. The authors believe that this is related to possible agglomeration of CeO_{2-x} particles upon an increase in their concentration.

Evidently, the mechanism of ROS inactivation by CeO_{2-x} nanoparticles is also applicable to biological systems; the self-regeneration ability of nanocrystalline cerium dioxide is also expected to be retained.

The luminescence spectra of cerium dioxide nanoparticles in the initial (partially reduced) state in the cells and biological liquids show a maximum at 451 nm.[52] After the reaction with ROS, this maximum shifts to 356 nm; however, after 5 to 20 min, the spectra return to the initial state with a maximum at 451 nm, which attests to regeneration of cerium dioxide nanoparticles.[52] Figure 11 shows the luminescence spectra of cerium dioxide nanoparticles that have been introduced *in vitro* into an astrocyte culture followed by addition of a solution of H_2O_2. In this case, the mechanism of action of cerium dioxide nanoparticles is similar to that for catalase. However, whereas the activity of CeO_{2-x} nanoparticles by the SOD mechanism depends on the content of cerium(III) on the surface, the activity by the catalase mechanism is caused by the presence of Ce^{4+} on the surface

$$2Ce^{4+} + H_2O_2 + 2OH^- \longrightarrow 2Ce^{3+} + 2H_2O + O_2.$$

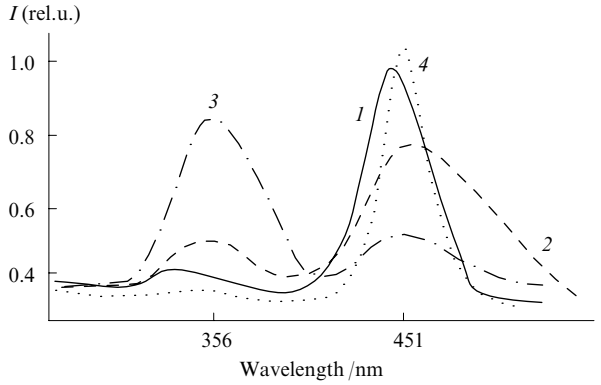


Figure 11. Luminescence spectra of cerium dioxide nanoparticles during H_2O_2 inactivation inside the cells.[52]
Without addition of H_2O_2 (*1*), with addition of H_2O_2: after 0.2 ms (*2*), 0.8 ms (*3*), 25 min (*4*).

The regeneration process of the CeO_{2-x} nanoparticle surface (Ce^{3+} oxidation) in aqueous H_2O_2 involves apparently the formation of cerium monoperoxy trihydroxide on the surface [reaction (1)] and has several rate-limiting steps the rates of which depend on a number of factors (hydrogen peroxide concentration, solution pH, *etc.*):

$$\begin{aligned} &Ce(OH)_3 + H_2O_2 \longrightarrow Ce(OH)_2OOH + H_2O, \\ &3\,Ce(OH)_2OOH + H_2O \longrightarrow 2\,Ce(OH)_3OOH + Ce(OH)_3, \\ &2\,Ce(OH)_3OOH \longrightarrow 2\,Ce(OH)_4 + O_2. \end{aligned} \qquad (1)$$

These data suggest that CeO_{2-x} nanoparticles can enhance cell survival under the action of H_2O_2. Indeed, experiments with a retinal neuron culture using fluorimetric analysis with 2,7-dichlorofluorescein [53] confirmed the protective action of cerium dioxide nanoparticles (Fig. 12).

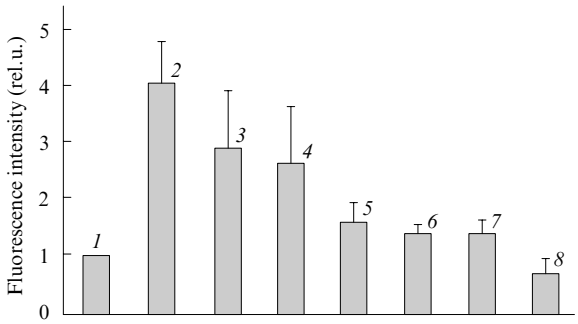


Figure 12. Inhibition of intracellular accumulation of ROS after introduction of 1 mmol litre^{-1} of H_2O_2 by pretreatment (12 h ahead) of retinal neurons with cerium dioxide nanoparticles.[53]
(*1*) Control run; (*2*) H_2O_2 without CeO_{2-x}; H_2O_2 and CeO_{2-x} in different concentration, nmol litre^{-1}: 1 (*3*), 3 (*4*), 5 (*5*), 10 (*6*), 20 (*7*); (*8*) CeO_{2-x} without H_2O_2.

The antioxidant action of nanodispersed CeO_{2-x} *in vitro* has been studied.[54,55] To study the protecting effect of CeO_{2-x} against oxidative stress, a suspension of CeO_{2-x} nanoparticles in a growth medium was added to the nerve cell line HT22, and after that, the cells were treated with glutamic acid, which induces their death by the oxytosis (apoptosis induced by oxidative stress).[56] The presence of cerium dioxide nanoparticles substantially prevented the cell death. According to the publication,[54] the number of cells that survived depends on the content of CeO_{2-x} but does not depend on the nanoparticle size. Note that more recent studies showed enhanced activity of CeO_{2-x} particles $\leqslant$20 nm in diameter. Additional experiments confirmed the mechanism of neuroprotective action of CeO_{2-x} related to the oxygen non-stoichiometry of this compound.[54]

The activity of cerium dioxide nanoparticles against oxidative stress in mammal cells has been studied.[57] For increasing the stability of CeO_{2-x} nanoparticle sols (3.7 nm), lecithin was used as the stabilizing agent. Stabilized particles were introduced into the cells βTC-tet of rat insulinoma, and the concentration of free radicals arising under the action of hydroquinone was determined. The results indicate that in the presence of cerium dioxide nanoparticles, the concentration of free radicals decreases. In addition, it was shown that CeO_{2-x} nanoparticles markedly enhance cell survival under oxidative stress.

Cerium dioxide nanoparticles provide protection from not only free radicals specially introduced into biological

cultures, but also from the ROS formed in biological systems under adverse conditions (mechanical injury, exposure to UV or ionizing radiation, *etc.*). This assumption was validated in a number of works;[53, 58–66] the mechanism of antioxidant action of CeO_{2-x} nanoparticles was also attributed to the presence of Ce^{3+} and Ce^{4+} ions on their surface.

In a study,[61] mixed organotypical brain cell cultures were injured *in vitro* (the method and conditions of injury were reported [62]). Figure 13 shows the action of CeO_{2-x} nanoparticles on the pre-injured brain cells. It follows from the data that in the presence of CeO_{2-x}, the vitality of neurons substantially increases. Thus, cerium dioxide nanoparticles can perform not only preventive, but also therapeutic function of protecting brain cells from traumatic stress.

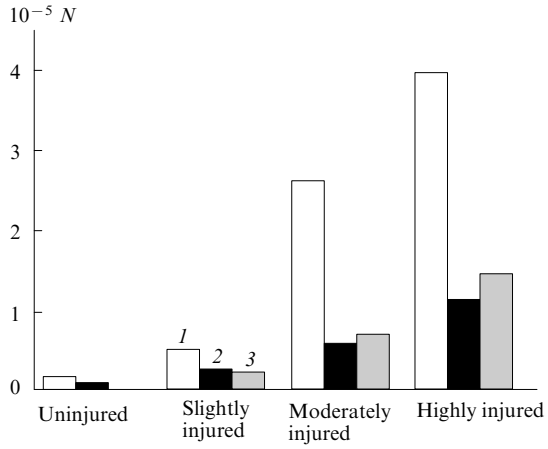


Figure 13. Death of brain neurons in the cultures treated with cerium dioxide nanoparticles (10 nmol litre^{-1}) and in control experiments.[61] (*1*) Control run, (*2*) CeO_{2-x} on the 10th day after *in vitro* injury; (*3*) CeO_{2-x} immediately after the damage. Hereinafter *N* is the number of dead cells per mg of the injuring protein.

Niua *et al.*[58] were the first to demonstrate *in vivo* that intravenously injected CeO_{2-x} nanoparticles reduce (owing to their antioxidant properties) the myocardial oxidative stress and cytoplasmic stress and suppress inflammatory processes in mice, thus ensuring protection against progression of cardiac dysfunction.

The protective properties of nanocrystalline cerium dioxide with respect to retinal neurons under their UV radiation were demonstrated.[53] Studies *in vitro* and *in vivo* have shown that pre-introduction of CeO_{2-x} into rat retina essentially prevents the death of the photoreceptor cells upon the subsequent exposure to an intense light flux; a CeO_{2-x} suspension with a concentration of 1 μmol litre^{-1} ensuring almost full protection of cells. According to electroretinography data, the functions of the retina are retained almost completely. The data on CeO_{2-x} introduction into the vitreous body after irradiation is of special interest. It was shown that this also enhances the survival of the photoreceptor cells. In the researchers' opinion,[53] the ability of cerium dioxide nanoparticles to directly bind ROS opens up the prospects of their use for preventing and treating the Alzheimer's, Parkinson's and Huntington's diseases.

These results were confirmed in another study.[64] Cerium dioxide nanoparticles were found to inactivate the free radicals and reactive molecules and prevent degenerative damages of rat retina. The authors assumed that cerium dioxide nanoparticles can be used to eliminate a series of factors that bring about blindness. This area is actively developed;[65] both the method for preparation of nanoparticles and the agent for retina protection were patented.[66]

Examples of the radioprotective action of cerium dioxide nanoparticles are given in Fig. 14. Mixed organotypic rat brain cells were obtained from newborn rats and cultivated by a reported procedure.[62] On the 10th day of *in vitro* cultivation, the cultures were treated with a solution (10 nmol litre^{-1}) of CeO_{2-x} nanoparticles. After 14–16 days *in vitro*, the cultures were exposed to UV radiation for 5 or 15 min and the damage caused by free radical was evaluated. For investigating the protection from γ-radiation, the cells were irradiated for 1 min (the absorbed dose was 1.5 or 5 Gy). Additionally, the CeO_{2-x}-treated cells were exposed to repeated UV or γ-radiation on the 68th day of cultivation to find out whether the protecting effect was retained. The results confirm that the cerium dioxide nanoparticles possess substantial radioprotective properties and can be used in the cancer therapy to protect the normal (benign) cells and to protect the organism from radiation damage.[63]

Note that on the 68th day of cultivation, all cells treated with CeO_{2-x} nanoparticles and not exposed to radiation remained vital, although such life span *in vitro* is not typical of a mixed culture of brain cells. In terms of the free radical theory of ageing, this fact suggests that cerium dioxide

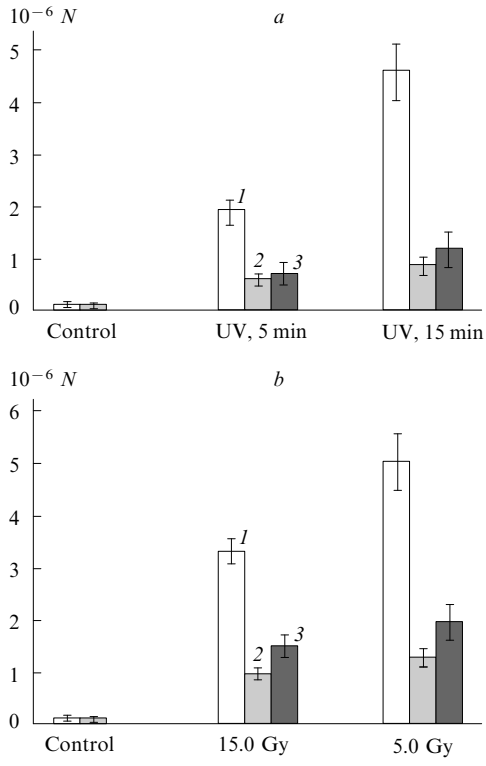


Figure 14. Effect of pretreatment of brain cell culture with cerium dioxide nanoparticles (10 nmol litre^{-1}) on cell death induced by UV radiation (*a*) and γ-radiation (*b*).[63]
(*1*) No pretreatment, (*2*) after 16 days *in vitro*, (*3*) after 68 days *in vitro* (for details, see the text).

nanoparticles can increase the life span of healthy somatic cells both in a culture and in macroorganisms. This suggestion was confirmed in a number of publications;[55, 61, 67, 68] the ability of cerium dioxide nanoparticles to extend the life of cells did not depend on the cell division ability. Published results[67] indicate that the introduction of CeO_{2-x} nanoparticles considerably increases the viability of some brain cells including astrocytes, neurons, microglial cells and oligodendrocytes. The cultures of these cells were shown to retain their morphological and biochemical characteristics for 6–8 months in the presence of nanodispersed cerium dioxide, whereas the life span of analogous cultures under usual conditions does not exceed 26–30 days. It was noted that this effect cannot be attained by using microcrystalline cerium dioxide or nanocrystalline powders of other metal oxides (including praseodymium, lanthanum, titanium and ruthenium).

Experiments for determining the effect of nanoparticles on the maximum lifetime of the organotypic brain cells were carried out.[61] On the 10th day of *in vitro* incubation, mixed cell cultures of rat cortex were treated with a colloid solution of cerium dioxide nanoparticles; the control sample was treated only with the buffer solution. The introduction of cerium dioxide nanoparticles in concentrations of 10–1000 nmol litre^{-1} increased the life span of brain cells many times. The efficiency of action depended on the particle size and composition.

The influence of nanodispersed cerium dioxide ($d = 3-5$ nm) on the survival of rat spinal cord cells was studied.[55] The introduction of CeO_{2-x} resulted in a pronounced decrease in the rate of loss of colony cells due to the clear-cut ability of cerium dioxide to bind free radicals. After neuron death, a characteristic enzyme, neuron-specific enolase (NSE), remains in the system. Figure 15 shows the dynamics of NSE evolution to the medium (in percent of the total amount of the enolase in the culture, in particular, in living neurons).[63] The massive loss of neurons and the maximum formation of NSE are observed under normal conditions on the 20 to 26th day, no living neurons being left in the system on the 30th day. In the presence of cerium dioxide nanoparticles, the content of NSE in the medium is not increased (remains at the base level), which indicates the survival of all neurons. In addition, it was shown that on treatment of neuron colonies with hydrogen peroxide, the fraction of surviving cells increases approximately threefold owing to the introduction of cerium dioxide nanoparticles. Presumably, the use of cerium dioxide in combination with other antioxidants can mitigate the consequences of spinal cord injury.

This feature of nanocrystalline cerium dioxide may prove useful for cultivation, storage or transportation *in vitro* of various cell cultures (including stem cell cultures).

The introduction of CeO_{2-x} nanoparticles may extend the life spans of not only micro- but also macroorganisms; in particular, the average and the maximum life spans of the fruit-fly *Drosophila melanogaster* were extended in this way (Fig. 16).[67, 68] The drosophilas received either standard food or the same food containing cerium dioxide nanoparticles in different concentrations; the nanoparticles were added to the food by ultrasonic homogenization technique. It is noteworthy that not only the maximum life span increased but also the time of halving of the population was longer for the colony that received the nanoparticles with food (see the dashed lines in Fig. 16). The additional nutrition by CeO_{2-x} nanoparticles also supports the normal geotaxis during ageing of drosophilas.[68]

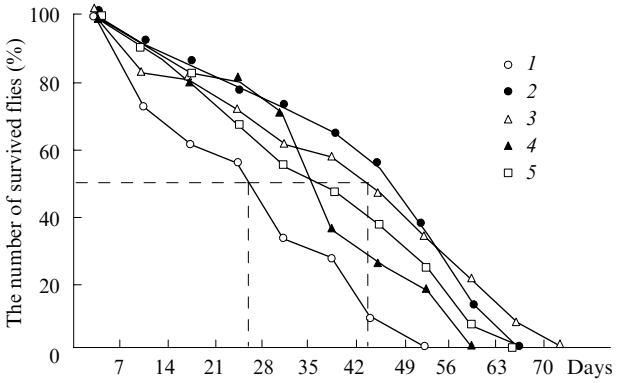


Figure 16. Effect of cerium dioxide nanoparticles on the life spans of the male flies *Drosophila melanogaster*.[68]
The concentration of CeO_{2-x} in the food of flies/nmol litre^{-1}: 0 (control run) (*1*), 1 (*2*), 10 (*3*), 100 (*4*), 1000 (*5*).

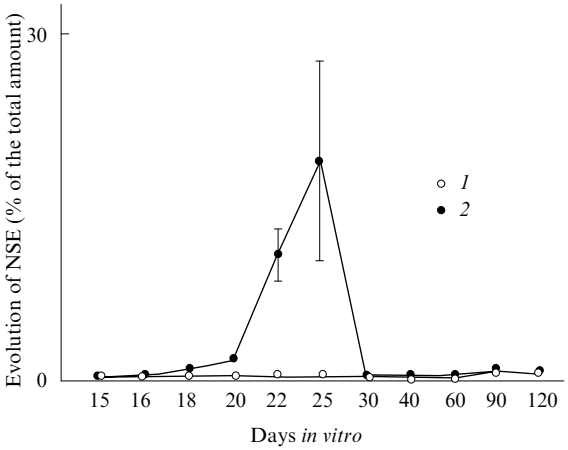


Figure 15. Evolution of neuron-specific enolase in the neuron cultivation medium in the presence of (*1*) and in the absence (*2*) of cerium dioxide nanoparticles.[63]

Cohen *et al.*[68] additionally demonstrated that CeO_{2-x} nanoparticles protect *Drosophila melanogaster* from the action of free radicals. The experiments were carried out using the agent Paraquat, which induces the insect death as a result of formation of free radicals and is widely used in biology to model the oxidative stress. Nanodispersed cerium dioxide (10 nmol litre^{-1}) was added directly to the food of flies during 35 days and after that the colony was placed for 24 h on a filter paper impregnated with Paraquat (20 mmol litre^{-1}) in a 5% solution of sucrose. Extension of both average and maximum life spans of the flies was noted. Similar results were obtained *in vitro* using nerve cell cultures treated with cerium dioxide nanoparticles and Paraquat.[63]

The introduction of copper-containing superoxide dismutase by genetic engineering technique into the drosophila DNA resulted in a statistically significant extension in the fly life span.[69] Meanwhile, after introduction of SOD into animal body by any other method, the enzyme is inactivated in the blood over a period of several minutes and has no

time to reach the area of generation of superoxide radicals in the cells (mitochondria).[70] Having the same key functions as SOD, cerium dioxide nanoparticles far surpass the enzyme in stability and can be introduced into the organism in different ways. Recall that in the drosophila experiments cerium dioxide was added directly to the food.

Owing to the unique ability of cerium dioxide nanoparticles to self-regeneration,[50, 71] they can participate in an infinite number of reduction – oxidation – reduction cycles [55] (unlike conventional antioxidants, for example, vitamins C and E). In particular, the protective actions of solutions of these vitamins and a sol of cerium dioxide nanoparticles under UV irradiation with respect to a brain cell culture were compared.[63] The results are shown in Fig. 17. It was also shown [56] that a single dose of cerium dioxide nanoparticles protects the cells from free radicals to a higher extent than a single or multiple use of vitamin E, *N*-acetylcysteine or melatonin. This feature of CeO_{2-x} may find use in the development of pharmaceutical compositions of systemic long-acting antioxidants with extended aftereffect.

One more practically valuable feature of cerium dioxide nanoparticles is that their antioxidant activity depends on pH of the medium. It was found [72] that CeO_{2-x} nanoparticles stabilized by dextran actively destroy hydrogen peroxide in neutral and alkaline solutions but are almost inactive in weakly acidic medium. We obtained analogous data on the regeneration of cerium dioxide nanoparticles of different size over a broad pH range after introduction of hydrogen peroxide (Fig. 18). It was shown that a decrease in pH entails a decrease in the extent of cerium dioxide reaction with H_2O_2 (measured as the change in the intensity of the CeO_{2-x} absorption band). The ability of particles to recover decreases in parallel. As the particle size decreases, the sensitivity to variation of the pH increases: $\sim$1 nm particles almost do not react with hydrogen peroxide at pH < 6, while larger particles react with H_2O_2 over the whole pH range studied.

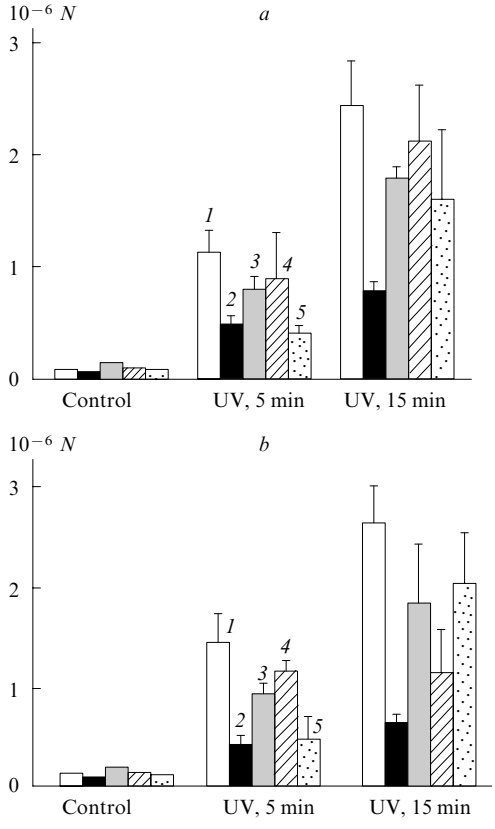


Figure 17. Effect of pretreatment with a single dose of cerium dioxide nanoparticles on the cell damage by free radicals (induced by UV radiation) as compared with a single dose (*a*) and multiple doses (*b*) of antioxidants: vitamin E (VitE), acetylcysteine (AC) and melatonin (Mel).[63]
(*1*) no pretreatment, (*2*) CeO_{2-x} pretreatment (10 nmol litre^{-1}), (*3*) VitE (100 μmol litre^{-1}), (*4*) AC (1 μmol litre^{-1}), (*5*) Mel (1 μmol litre^{-1}).

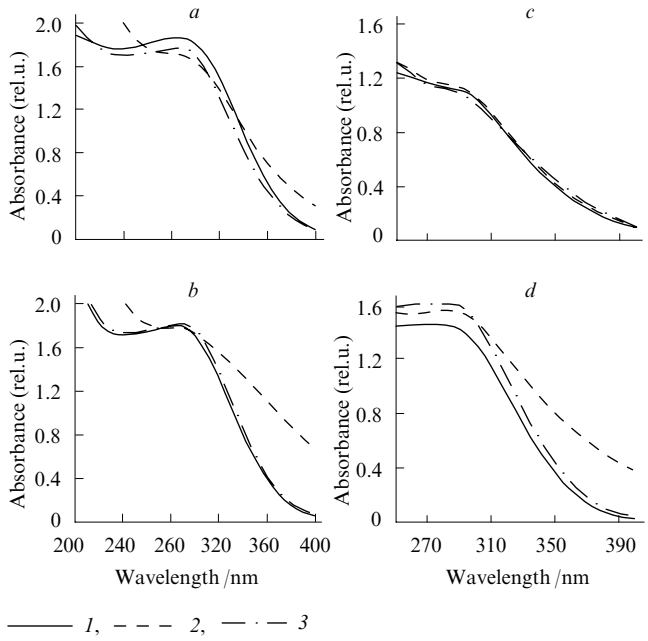


Figure 18. Regeneration of CeO_{2-x} nanoparticles stabilized by sodium citrate (*a*, *b*) and polyacrylate (*c*, *d*) in alkaline (pH 9) (*b*, *d*) and acidic (pH 4) (*a*, *c*) media after treatment with hydrogen peroxide. (*1*) Initial solution of CeO_{2-x} (0.5 mmol litre^{-1}), (*2*) after addition of H_2O_2 (1.0 mmol litre^{-1}), (*3*) 7 days after introduction of H_2O_2.

The pH value is known to decrease (< 7) in the vicinity of malignant tumours; thus, the dependence of the antioxidant activity of CeO_{2-x} nanoparticles on the medium acidity should be manifested in the inactivation of ROS around the tumour. Indeed, this assumption was confirmed [72] using destruction of cardiomyocytes, fibroblasts and carcinoma cells by hydrogen peroxide as an example (Fig. 19): the presence of CeO_{2-x} nanoparticles protected normal cells and simultaneously did not interfere with the H_2O_2-induced death of tumour cells. This marks a new trend of the use of cerium dioxide nanoparticles in cancer therapy.

The physicochemical behaviour of cerium dioxide nanoparticles in the organism deserves special investigation. By means of electron microscopy, microspectrophotomertry and inductively coupled plasma mass spectrometry (ICP MS), it was demonstrated [63] that < 20 nm cerium dioxide nanoparticles easily penetrate cells *in vitro* and cells of living organisms *in vivo*. Doses 100-fold higher than those needed for extending the life span of the cell culture were

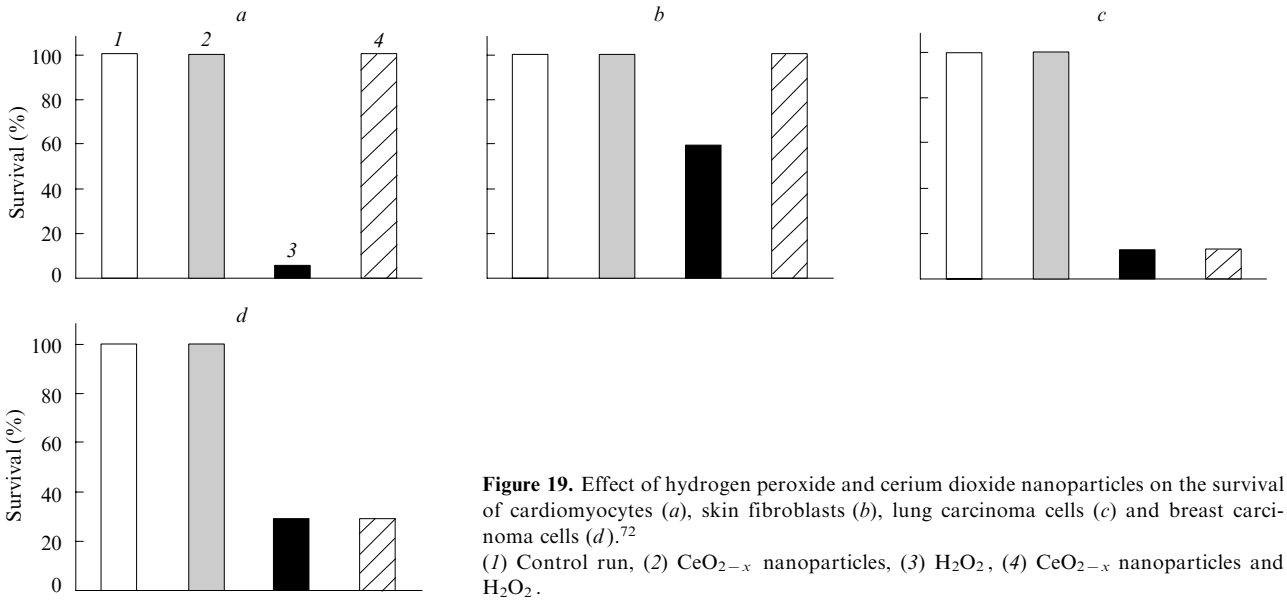


Figure 19. Effect of hydrogen peroxide and cerium dioxide nanoparticles on the survival of cardiomyocytes (*a*), skin fibroblasts (*b*), lung carcinoma cells (*c*) and breast carcinoma cells (*d*).[72]
(*1*) Control run, (*2*) CeO_{2-x} nanoparticles, (*3*) H_2O_2, (*4*) CeO_{2-x} nanoparticles and H_2O_2.

non-toxic for drosophilas. Single-shot injections of nanoparticles (0.3–3 mmol litre^{-1}) into the mouse tail vein did not cause any organic damage or behavioural deviations.[63] Cerium dioxide nanoparticles were found to be mainly accumulated in the brain, heart and lungs with low excretion for 6 months. The cerium content in tissues is approximately twice higher than the background level but remains within hundreds μg kg^{-1} (ppb).

The ICP MS measurements of the concentration of cerium dioxide nanoparticles in various organs of mice of the line Balb/c within 5 days after the injection of 5–10 μl of a CeO_{2-x} solution (concentration 300 nmol litre^{-1}) into the tail vein confirmed the accumulation of cerium in the tissues of the above-listed organs. In the cells, CeO_{2-x} nanoparticles are located near mitochondria, which are the main sources of ROS.[59]

Takaya [73] analyzed the solubility of submicrone CeO_{2-x} particles in deionized water, in brine and in the Gamble solution, which mimics extracellular fluid and contains chloride, phosphate and carbonate ions and some organic acid salts. It was found by ICP MS that the concentration of cerium ions after keeping CeO_{2-x} in the Gamble solution for a week is not more than several tens of μg kg^{-1}.

The accumulation dynamics of CeO_{2-x} nanoparticles in the fibroblasts of human lungs was investigated.[74] It was shown that nanoparticles are fully localized in lipid bilayers. The content of cerium dioxide nanoparticles in the cells increases with time according to a linear law; the rate of CeO_{2-x} accumulation increases with increase in the nanoparticle size from 20–50 to 250–500 nm, which is caused by substantial aggregation of smaller particles due to the adsorption of proteins of their surface.

Systematic studies of cerium dioxide nanoparticles with 9 and 320 nm size carried out by Park *et al.*[75] using standard *in vitro* tests for skin irritation, cytotoxicity, mutagenicity, *etc.* indicate that nanodispersed CeO_{2-x} does not have significant adverse effect on living systems. Cerium dioxide nanoparticles 6–12 nm in diameter were found to possess no cytotoxicity with respect to nerve cell line HT22 and mouse macrophage line RAW164.[54] This fact is especially important, for example, due to the fact that CeO_{2-x}-based catalysts are already widely used as diesel fuel additives and thus can enter the environment in considerable amount.[76]

According to our data (obtained in cooperation with the Division of Interferons and Immunomodulators of the Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine), cerium dioxide nanoparticles in concentration of $\leqslant$100 mg litre^{-1} are non-toxic for the epithelial cells of transplantable porcine testicle cells and mouse fibroblasts. The lack of toxicity of CeO_{2-x} nanoparticles with respect to prokaryotic cells of microorganisms was confirmed (in cooperation with the A N Marzeev Institute of Hygiene and Medical Ecology of the Ukrainian Academy of Medical Sciences). However, in another work [77] it was found that nanodispersed cerium dioxide is cytotoxic with respect to the human lung cancer cell line A549; the cell viability significantly decreased upon increase in the concentration of CeO_{2-x} suspensions. Note that in this case, the introduction of cerium dioxide decreased the level of antioxidants (glutathione and α-tocopherol), which was attributed to generation of free radicals on the CeO_{2-x} particle surface. This conclusion is at variance with the results of a number of publications [53–55, 67] and requires verification. According to our data (obtained in cooperation with the R E Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of the National Academy of Sciences of Ukraine), cerium dioxide nanoparticles stabilized by sodium citrate are non-toxic over a broad concentration range with respect to both normal donor lymphocytes and malignant lymphocytes.

The action of an aqueous dispersion of CeO_{2-x} nanoparticles (7 nm) on gram-negative bacteria *Escherichia coli* has been studied.[50] It was shown that in neutral medium, CeO_{2-x} nanoparticles are positively charged and, hence, they are electrostatically attracted to bacterial outer membranes.[78] By counting of colony-forming units after direct contact with cerium dioxide nanoparticles, conditions for CeO_{2-x} toxicity with respect to *E. coli* were elucidated. The toxicity was manifested in aqueous solution but not in the growth medium.

Our studies of the action of nanocrystalline cerium dioxide on the genetically engineered *E. coli* strain TG1 the

bioluminescence intensity of which correlates with the enzymatic activity of bacteria have shown [79] that up to the highest concentration (20 000 mg litre^{-1}), suspensions of CeO_{2-x} in distilled water are non-toxic with respect to the test culture. It was first found that the luminescence intensity of bacteria (both in the presence and in the absence of cerium dioxide) decreases with time; however, the introduction of CeO_{2-x} into cell cultures sharply retards the inhibition of bioluminescence. Figure 20 shows the normalized (I/I_0) time dependences of the luminescence intensity, which indicate that a decrease in the CeO_{2-x} particle size leads to a pronounced increase in the bioactivity of cerium dioxide powder. The observed influence of the size factor on the bioactivity of CeO_{2-x} nanopowders can be attributed to the above-described increase in the bulk and surface nonstoichiometry of CeO_{2-x} nanoparticles following a decrease in their size, which makes them more reactive with respect to ROS.

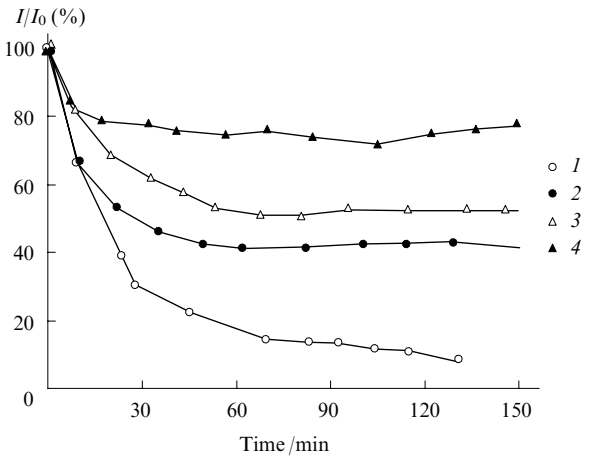


Figure 20. Relative bioluminescence intensity of the bacteria *Escherichia coli* TG1 in the presence of CeO_{2-x} particles.[79]
Particle size, nm: 8 (*1*), 6 (*2*), 5 (*3*), < 5 (*4*).

In view of the set of unique properties of nanocrystalline cerium dioxide, specifically, regeneration capability, protection from free radicals *in vitro* and *in vivo*, increase in the life spans of biological objects (cells and whole organisms), it was proposed [63, 71, 80] to distinguish a new area in medicine, radical nanomedicine, and to call the agents like CeO_{2-x} nanopharmaceutical agents.[81]

As soon as 2008, medicinal agents based on cerium dioxide nanoparticles were proposed [82, 83] for treating diabetic ulcers; the authors noted low toxicity of CeO_{2-x} with respect to neutrophiles and macrophages. This provided a high degree of protection of cells from the oxidative-stress induced death.

The agents based on cerium dioxide nanoparticles can be administered in different ways.[63] These include infusions and intramuscular, intraperitoneal, subcutaneous or intrathecal injections. Other examples propose external application as lotions, balms or bandages applied on intact skin or open wounds. Administration through mucous membranes is also possible, *e.g.*, intranasally by inhalation of dry particles or a fume incorporating the particles, orally together with a meal, sublingually, rectally and so on. Any dosage form is possible including solutions, gels, tablets, capsules, powders, creams, lotions, balms, inhalers and so on.

The amount of cerium dioxide nanoparticles prescribed in each particular case depends on usual factors that are taken into account when dosing pharmaceutical agents (patient weight, general health and metabolic activity). In the general case, the prescribed amount varies from 10 ng to 1 g per single dose or unit body weight (kg); the amount of the agent used for injection or infusion should be closer to the lower limit of this range, while in the case of oral administration the highest amount may be allowed. The currently available results suggest that the optimal dose for ~20-nm cerium dioxide nanoparticles should provide a concentration in blood or intracellular fluids at a level from about 10 nmol litre^{-1} to about 1 μmol litre^{-1} of nanoparticles (about 1.5–150 μg litre^{-1} in relation to CeO_{2-x}).

IV. Key methods for the preparation of nanodispersed cerium dioxide and nanopharmaceutical agents based thereon

The most widely used methods of the preparation of ultra- and nanodispersed cerium dioxide powders include the methods based on precipitation of this compound in the hydrated form from solutions of cerium(III) salts or cerium(IV) compounds induced by the addition of strong bases. Since cerium(IV) salts are either poorly soluble or susceptible to hydrolysis even at low pH values, cerium(III) salts are used most often for preparing cerium dioxide nanoparticles from aqueous solutions. The solubility products of cerium(III) hydroxide and cerium dioxide are extremely low, therefore, precipitation occurs under highly non-equilibrium conditions. Using a number of examples, we demonstrated that these conditions of precipitation often rule out the possibility of targeted variation of the particle size (3–5 nm) for the solid products but allow one to govern the character of nanoparticle aggregation and thus their specific surface area.[84–87] In particular, it was found that [85] the fractal dimension of the surface (2.50–2.68) and the specific surface area (110–220 m^2 g^{-1}) of CeO_{2-x} powders synthesized from aqueous ethanol solutions of $Ce(NO_3)_3$ are determined by the water : ethanol ratio in the starting solution.

The use of reagents (for example, urea and hexamethylenetetramine) that are slowly hydrolyzed in aqueous solutions to give hydroxide ions appears to be a a successful alternative to fast precipitation of CeO_{2-x}; this allows one to control the degree of supersaturation and avoid the formation of local concentration gradients, which inevitably arise upon usual mixing of solutions containing a cerium salt and a precipitating agent. This method provides cerium dioxide nanoparticles with specified size ranging from 6 to 50 nm.[88–93] According to our data,[87, 92] homogeneous hydrolysis of cerium(III) salts in the presence of hexamethylenetetramine can also be used for the synthesis of ultrathin (< 1 nm thick) CeO_{2-x} nanoplates having large anisotropy coefficients (~50 : 1). The size of the CeO_{2-x} nanoparticles thus formed depends on the synthesis temperature and duration, concentration, reactant ratio, and other factors.

Additional control over the size and shape of CeO_{2-x} particles can be provided by using hydrothermal (including hydrothermal microwave) treatment of suspensions of nanocrystalline cerium dioxide. The micromorphology of CeO_{2-x} was found [94–102] to depend on the temperature and

duration of the hydrothermal synthesis, the pressure in the hydrothermal reactor, the presence of mineralizing agents (alkali and alkaline metal salts) and medium pH and the precipitation conditions of the initial suspensions of CeO_{2-x}. In particular, the hydrothermal treatment in highly alkaline conditions is among the few methods that provide highly anisotropic cerium dioxide nanorods.[87,102]

It is noteworthy that metered application of cerium dioxide-based agents usually requires durable CeO_{2-x} sols stable against aggregation; this brings about the necessity of development of methods for their synthesis. As a rule, for increasing the solubility and stability of colloid solutions of CeO_{2-x} nanoparticles, various additives and coatings are used. Thus polyaniline-coated CeO_{2-x} nanoparticles were produced[103] by direct oxidation of aniline by cerium dioxide; the nanoparticle surface contained mainly Ce^{3+}. Cerium dioxide nanoparticles were also obtained[47] by a sonochemical technique using tetraalkylammonium hydroxide as the stabilizing agent. However, for biological purposes, cerium dioxide nanoparticles stabilized by polyaniline or quaternary ammonium compounds are of only theoretical interest due to the high toxicity of these stabilizing agents.

Methods for the synthesis of colloid cerium dioxide particles in reverse microemulsions were proposed.[104–106] The heptane–aqueous $CeCl_3$ [or $Ce(NO_3)_3$] system stabilized by AOT [sodium bis(2-ethylhexyl)sulfosuccinate], DDAB [bis(dodecyl)dimethylammonium bromide] or a mixture of DDAB with Brij35 (monododecyl ether of tricosaethylene glycol) was used.[104,105] Micellar solutions of DTAB (dodecyltrimethylammonium bromide) and vesicular DDAB systems were used as media for the preparation of cerium dioxide. This afforded virtually non-agglomerated nano-sized (6–13 nm) CeO_2 crystallites, whereas without a surfactant, the crystallite size reached 21 nm. Lee *et al.*[106] described the synthesis of CeO_{2-x} by mixing two emulsions in kerosene, one containing an aqueous solution of the precursor (cerium nitrate) and the other containing an aqueous solution of sodium hydroxide. The cerium dioxide particles thus formed were nearly spherical and their size (10–20 nm) was determined by the concentration of reactant solutions.

A patent[107] combines the redox and micellar processes for the preparation of cerium dioxide nanoparticles. AOT was dissolved in a non-polar solvent (toluene), and an aqueous solution of cerium(III) nitrate was added with intense stirring (homogenization). Micelles representing surfactant-stabilized microdrops of the aqueous solution of the cerium salt were formed in the toluene bulk. Note that these stages of micellar preparation of nanoparticles are used in all procedures, the differences being only in the way of the subsequent stimulation of the formation of CeO_{2-x} particles in the micelle bulk. Usually this is attained by increasing the pH, heating, sonication, *etc*. Hydrogen peroxide was used[105] for treating the micellar system, which induced the $Ce^{3+} \rightarrow Ce^{4+}$ oxidation, nucleation and nanoparticle growth. The stable solution of monodispersed cerium dioxide nanoparticles thus formed can be subjected to pyrolysis to form CeO_{2-x} nanopowders, used as such or transformed to the aqueous phase by replacing the solvent. Unfortunately, in this case, too, biologically inadmissible stabilizing agents were used.

A considerable number of methods for the production of nanopowders and colloid solutions of cerium dioxide have been patented.[107–116] Most are based on the synthesis from aqueous and nonaqueous solutions of cerium compounds at relatively low temperatures. Thus in a patent,[110] a highly concentrated (up to 600 g litre^{-1}) sol with an average particle diameter of 50 nm stabilized by nitric acid was prepared by purification repeated many times, dialysis and redispersion of cerium dioxide nanoparticles synthesized by hydrothermal method. A method for stabilization of cerium dioxide nanoparticles with acetic acid and ammonium acetate was patented.[111] The patents[112,113] propose preparing of nanocrystalline CeO_{2-x} by direct hydrolysis of cerium salts with ammonia followed by drying or annealing of the precipitate and redispersion in water. In another patent,[114] it is proposed to introduce a polyoxyalkene polymer (4–10 oxyethyl units) with terminal phosphonate groups into the product obtained as described in the earlier patent.[113] This modification of the nanoparticle surface extends the stability range of the CeO_{2-x} sol to pH 10. Colloid solutions of cerium dioxide nanoparticles (~2 nm in diameter, pH 2, concentration 200 g litre^{-1}) were also stabilized using acetylacetone, salicylic acid and the surfactant Rhodiastab 50® [$PhC(O)CH_2C(O)C_{17}H_{35}$-n]. This gave rise to a sol stable in neutral and weakly alkaline media.[115]

The topical task of synthesis of water-soluble biocompatible cerium dioxide nanoparticles was outlined.[50] To this end, Tsai *et al.*[57] used lecithin devoid of cytotoxic properties; the resulting CeO_{2-x} nanoparticles with a size of 3.5 nm were stable in a buffer solution of trisodium citrate.

Note that citric acid is one of the most promising components of buffer solution or stabilizing agent as regards biocompatibility. We have developed and now successfully use a procedure where trisodium citrate serves as the only stabilizing agent for cerium dioxide nanoparticles. This procedure comprises hydrolysis of a solution of cerium(IV) salt in a highly alkaline medium (pH > 12), the formation and sedimentation of CeO_{2-x} nanoparticles, washing of the precipitate to remove by-products (electrolytes) repeated many times to reach a neutral pH value, ultrasonic redispersion of nanoparticles in the presence of an equimolar amount of a stabilizing agent, addition of an excess of hydrogen peroxide and refluxing of the sol thus formed until traces of peroxy compounds are removed. The citrate ions are specifically adsorbed on the surface of cerium dioxide nanoparticles to form an electric double layer, which provides the required aggregative stability of the sols. Thus it is possible to prepare nanocrystallites of CeO_{2-x} that are distinguished by ultrasmall size ($\leqslant$2 nm), are stable in water, buffer solutions and physiological fluids over a broad pH range (from 1 to 12) and do not show signs of sedimentation on prolonged storage. We also proposed a method for the preparation of sedimentation-stable aqueous sols of CeO_{2-x} based on a combination of anion-exchanger and hydrothermal microwave treatment of solutions of cerium(III) nitrate. This method allows one to control the CeO_{2-x} nanoparticle size within 3.5–8 nm, the sols thus formed remaining stable even after they have been kept in the autoclave at 190 °C, which ensures the possibility of their reliable sterilization.

A biologically compatible polymer, dextran, was also used as the stabilizing agent.[72] The nanoparticles thus obtained comprised a cerium dioxide core 4 nm in diameter surrounded by a dextran shell and had an overall size of ~10 nm.

Chicken egg albumin has also been used as the stabilizing agent.[117] Unfortunately, characteristics of the gel con-

taining CeO_{2-x} nanoparticles obtained in this way vary on storage and this product is of no practical value. Non-toxic polyvinylpyrrolidone (PVP) has also been used to stabilize cerium dioxide nanoparticles.[118] The CeO_{2-x} particle size depended on the molecular mass of the polymer introduced during the synthesis; the advantages of this method include the possibility to concentrate the $PVP-CeO_2$ system by evaporation and redisperse. Note that PVP is sorbed non-specifically on the surface of cerium dioxide particles and does not provide a sufficient decrease in the surface free energy for the formation of small-size nanoparticles. The resulting sol of coarse 50–120 nm nanoparticles is of low utility for biomedical applications; in the authors' opinion, the system can be rather of interest for the preparation of photonic crystals.

The use of cerium dioxide nanoparticles for biomedical purposes requires development of methods not only for their stabilization but also for their targeted delivery to damaged organs (and, preferably, for visualization); this brings about the task of their additional functionalization. One of the first studies along this line[80] proposed a method for the synthesis of functional cerium dioxide nanoparticles based on successive modification of the surface of CeO_{2-x} particles by epichlorohydrin and carboxybenzenesulfamide; the latter inactivates the enzyme hCAII (carboanhydrase), which promotes the development of glaucoma. In the same work, in addition to the carboanhydrase inhibitor, a fluorophor (carboxyfluorescein) was grafted to the nanoparticle surface; this allowed tracing the behaviour of CeO_{2-x} nanoparticles *in vitro* and *in vivo*.

A method for the synthesis of aqueous sols of CeO_{2-x} that we proposed[119] makes use of non-toxic short-chain polyacrylic acid (PAA) as the stabilizing agent. The sols of cerium dioxide nanoparticles thus obtained are able to fluoresce on exposure to UV radiation (this provides visualization of the nanoparticles), and the acrylic end groups can be used for the subsequent targeted functionalization. The CeO_{2-x} nanoparticles formed are ultrasmall ($\sim$1 nm), stable in water and buffer solutions over a broad pH range and in physiological fluids. It is of crucial importance that the polyacrylate monolayer does not prevent the access of ROS (including free radicals) to the nanoparticle surface. The redox properties of the CeO_{2-x} nanoparticles formed depend on the pH; hence, CeO_{2-x}–PAA sols are promising for the radiation therapy of tumours.

* * *

As a conclusion, it should be emphasized that the scope of application of nanocrystalline cerium dioxide is far from being restricted to the examples presented in this review. Cerium dioxide and materials based thereon are parts of various catalysts, corrosion protective coatings of metals and alloys, polishing formulas and grinding materials (including those for chemicomechanical surface treatment of silicon plates in micro- and nanoelectronics), antireflection coatings of solar cell batteries, sun-protection cosmetic agents, electrochromic devices, sensors and biosensors, *etc*. This broad spectrum of conventional and prospective applications of cerium dioxide, including the biomedical applications considered here is related to the unique set of physicochemical properties of this compound, first of all, with the clear-cut effect of the size factor on the oxygen nonstoichiometry of CeO_{2-x}. This is responsible for the high biological activity of nanocrystalline cerium dioxide based on binding of reactive oxygen compounds and radicals deleterious for living systems. However, the body of experimental knowledge of the physicochemical and biological properties of nanodispersed cerium dioxide is still relatively limited. Therefore, development of scaled processes for the preparation of CeO_{2-x} with controlled particle size distribution, studies of the structure of nanocrystalline CeO_{2-x} (in particular, with the use of modern neutron and synchrotron radiation sources) and development and clinical testing of biologically active agents containing cerium dioxide nanoparticles are quite topical tasks.

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