

Improving ·OH scavenging properties of nanoceria by doping and pre-irradiation

*V.V.Seminko, P.O.Maksimchuk, O.O.Sedyh,
A.V.Aslanov, Yu.V.Malyukin*

Institute for Scintillation Materials, STC "Institute for Single Crystals",
National Academy of Sciences of Ukraine,
60 Nauky Ave., 61072 Kharkiv, Ukraine

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Hydroxyl radicals (·OH) are usually considered as the most dangerous type of reactive oxygen species formed inside the living cells that leads to increasing demand for antioxidant nanomaterials able to effectively ·OH elimination. Nanoceria (CeO_{2-x}) has recommended itself as the one of the most potent ·OH scavengers due to high content of Ce^{3+} ions and easy $\text{Ce}^{3+} \leftrightarrow \text{Ce}^{4+}$ switching making possible effective redox cycling. In the paper the direct connection between Ce^{3+} content and ·OH scavenging ability is shown. Doping of nanoceria by non-isovalent ions (Y^{3+}) or by ions with smaller ionic radius (Zr^{4+}) leads to increase of both Ce^{3+} content and antioxidant activity of nanoparticles. The same increase of ·OH scavenging ability is observed at pre-irradiation of nanoceria ($\lambda = 325$ nm) which is accompanied by transfer of the part of Ce^{4+} ions to Ce^{3+} ones. The observed effects are caused by formation of additional oxygen vacancies at doping or pre-irradiation providing increase in the number of sites ($\text{Ce}^{3+}\text{-Ov-Ce}^{3+}$ or $\text{Ce}^{3+}\text{-Ov-RE}^{3+}$) for ·OH scavenging.

Keywords: nanoceria, hydroxyl radicals, luminescence, pre-irradiation.

Гидроксил-радикалы (·OH) обычно рассматриваются как наиболее опасный тип активных форм кислорода, образующихся в живых клетках, что приводит к увеличению потребности в антиоксидантных наноматериалах, способных эффективно удалять ·OH. Нанокристаллический оксид церия (CeO_{2-x}) зарекомендовал себя как один из наиболее эффективных материалов для борьбы с гидроксил-радикалами благодаря высокому содержанию ионов Ce^{3+} и способности к переключению $\text{Ce}^{3+} \leftrightarrow \text{Ce}^{4+}$, что обуславливает способность оксида церия к саморегенерации. Показана прямая связь между содержанием ионов Ce^{3+} и способностью оксида церия уничтожать гидроксил-радикалы. Введение в оксид церия неизоэлектронных ионов (Y^{3+}) или ионов с меньшим ионным радиусом (Zr^{4+}) приводит к увеличению как содержания Ce^{3+} , так и антиоксидантной активности наночастиц. Такое же увеличение антиоксидантного действия наблюдается при предварительном лазерном облучении оксида церия ($\lambda = 325$ нм), которое сопровождается переходом части ионов Ce^{4+} в ионы Ce^{3+} . Наблюдаемые эффекты обусловлены образованием дополнительных кислородных вакансий при введении примесных ионов или предварительном облучении, обеспечивающих увеличение числа комплексов $\text{Ce}^{3+}\text{-Ov-Ce}^{3+}$ или $\text{Ce}^{3+}\text{-Ov-RE}^{3+}$, способных к уничтожению гидроксил-радикалов.

Поліпшення антирадикальних властивостей нанокристалів оксиду церію при введенні домішкових іонів та попередньому опроміненні. *В.В.Семінько, П.О.Максимчук, О.О.Седих, А.В.Асланов, Ю.В.Малюкін.*

Гідроксил-радикали (·OH) зазвичай розглядаються як найбільш небезпечний тип активних форм кисню, що утворюються у живих клітинах. Це призводить до збільшення потреби в антиоксидантних наноматериалах, здатних ефективно видаляти ·OH.

Нанокристалічний оксид церію (CeO_{2-x}) зарекомендував себе як один з найбільш ефективних матеріалів для боротьби з гідроксил-радикалами завдяки високому вмісту іонів Ce^{3+} і здатності до переключення Ce^{3+} і Ce^{4+} , яке зумовлює здатність оксиду церію до саморегенерації. Показано прямий зв'язок між вмістом іонів Ce^{3+} і здатністю оксиду церію знищувати гідроксил-радикали. Введення в оксид церію неізовалентних іонів (Y^{3+}) або іонів з меншим іонним радіусом (Zr^{4+}) приводить до збільшення як вмісту іонів Ce^{3+} , так і антиоксидантної активності наночастинок. Таке покращення антиоксидантної дії спостерігається також при попередньому лазерному опроміюванні оксиду церію ($\lambda = 325$ нм), яке супроводжується переходом частини іонів Ce^{4+} в іони Ce^{3+} . Ефекти, що спостерігаються, обумовлені утворенням додаткових кисневих вакансій при введенні домішкових іонів або попередньому опроміненні, що забезпечує збільшення числа комплексів $\text{Ce}^{3+}\text{-Ov-Ce}^{3+}$ або $\text{Ce}^{3+}\text{-Ov-RE}^{3+}$, здатних до знищення гідроксил-радикалів.

1. Introduction

Reactive oxygen species (ROS) such as O_2^- (superoxide anions), H_2O_2 (hydrogen peroxide), and $\cdot\text{OH}$ (hydroxyl radicals) are biologically active molecules which are formed inside the mitochondria of living cells during cellular respiration due to leakages in electron transport chain [1]. Some ROS play an important and indispensable role in the metabolism of the cell like H_2O_2 which role as a cell messenger was recently recognized in the number of studies [2, 3]. At the same time, an increase of the content of hydroxyl radicals which are the strongest oxidants among all ROS can trigger the number of pathological processes inside the cell from enhanced lipid peroxidation of cell membranes to DNA damage [4]. Hydroxyl radicals are formed at water radiolysis during X-ray or gamma-irradiation of the cell ($\text{H}_2\text{O} \rightarrow \text{H} \cdot + \cdot\text{OH}$), or can be effectively produced from hydrogen peroxide in the presence of Fe^{2+} ions via Fenton reaction ($\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \cdot\text{OH} + \text{OH}^-$) [5]. Extremely high reactivity of $\cdot\text{OH}$ radicals (average $\cdot\text{OH}$ lifetime in the biological environment of only few nanoseconds) makes the task of its effective elimination by the internal systems of the living cell rather difficult.

The ability of cerium oxide nanoparticles (nanoceria) to effective antioxidant defense of the living cells have been discussed in the number of publications since the first observation of their ROS-protective action in 2006 [6]. The unique enzyme-like action of nanoceria enables long-term protection of the cells against excessive concentrations of superoxide (SOD-like activity [7]) and hydrogen peroxide (catalase-like activity [8]). In [9] the $\cdot\text{OH}$ scavenging action of nanoceria was observed during study of the oxidation of methyl violet by the mixture of Fe^{2+} and H_2O_2 with and without ceria nanoparticles. The authors [9] have also ob-

served a clear dependence of antioxidant activity on the size of nanoparticle being higher for smaller NPs than for bigger ones. As the reduction of the size of ceria NP is accompanied by increase of Ce^{3+} content, they have concluded that $\cdot\text{OH}$ scavenging activity of nanoceria is directly proportional to the number of Ce^{3+} ions on the surface of NP. Later, other authors [10] have shown that besides $\cdot\text{OH}$ scavenging nanoceria can also generate additional $\cdot\text{OH}$ radicals via reaction similar to Fenton reaction. Overall, the way nanoceria behaves in the biological or model system depends strongly on the properties of nanoparticle itself as well as on the properties of the environment (for instance, pH).

In this paper we consider both the interconnection between $\cdot\text{OH}$ scavenging and $\text{Ce}^{3+}/\text{Ce}^{4+}$ ratio in nanoceria, and the ways of improving $\cdot\text{OH}$ scavenging ability by increase of Ce^{3+} content. The influence of doping nanoceria by non-isovalent (Y^{3+}) or smaller (Zr^{4+}) ions and pre-irradiation of nanoparticles on the Ce^{3+} content and $\cdot\text{OH}$ scavenging properties of nanoceria is discussed.

2. Experimental

Colloidal water solutions of nanoceria were obtained using the methods of colloidal synthesis. 100 ml of solution containing CeCl_3 (0.007 mol/L) and YCl_3 or ZrOCl_2 (0.0008 mol/L) was mixed with 100 ml of solution of sodium citrate $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$, then was added 200 ml hexamethylenetetramine (0.08 mol/L) and stirred by using a magnetic stirrer for 3 h at room temperature. Then, the solution was heated at 60°C for 1.5 h. As a result, transparent solution with a yellow color was obtained. After that, 0.7 ml of solution NH_4OH and 0.7 ml of H_2O_2 (60 %) were added into the solution. Then, the solution was put in round-bottom flask and was refluxed for 5 h. A

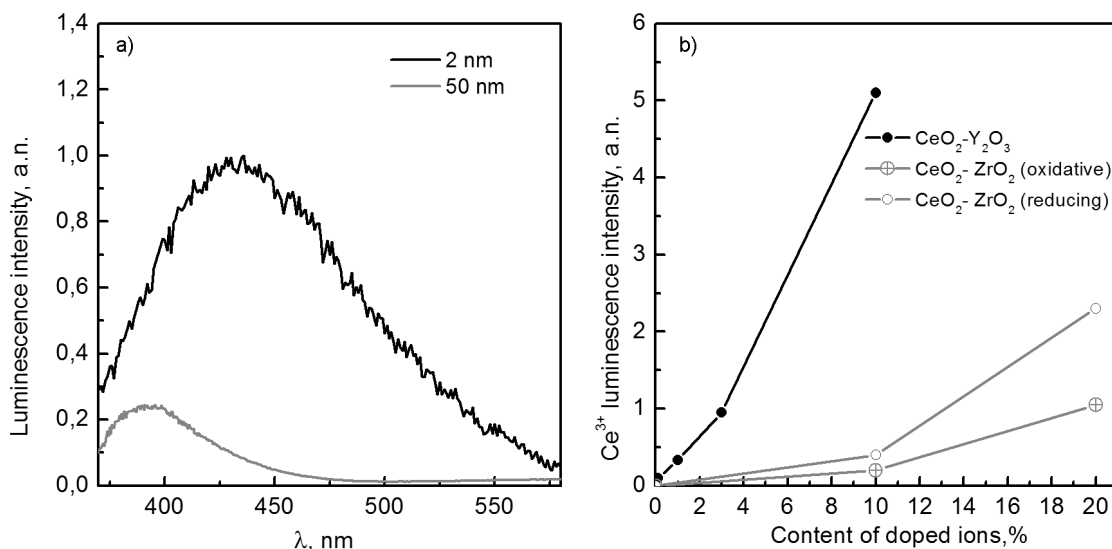


Fig. 1. a) Luminescence spectra of 2 nm and 50 nm nanoceria (the latter after treatment in reducing atmosphere), $\lambda_{exc} = 325$ nm; b) Dependence of Ce^{3+} luminescence intensity on the content of doped ions in Y^{3+} - and Zr^{4+} -doped 50 nm nanoceria.

solution of 1 M $CaCl_2$ was added to the obtained solution until the resulting solution became turbid. Then the solid phase was precipitated by centrifugation. The precipitate was separated and a solution of $CaCl_2$ was added again. The procedure of precipitate cleaning was repeated three times. After the last stage of centrifugation, a solution of $Na_3C_6H_5O_7$ with molar ratio $CeO_2:Y(Zr)/Na_3C_6H_5O_7$ as 1:1 was added to the precipitate. The sol was filtered through membrane filters with pores of 200 nm.

Luminescence spectra of nanoceria were excited by a continuous-wave GKL-4UM He-Cd laser ($\lambda = 325$ nm) and registered using the SDL-1 grating monochromator with the Hamamatsu R9110 PMT in the photon counting mode. Influence of nanoceria on the processes of $\cdot OH$ generation at X-ray irradiation of water solutions was determined using standard coumarin test. Intensity of coumarin fluorescence (at $\lambda_{exc} = 325$ nm) for X-ray irradiated nanoceria water solutions was compared with coumarin fluorescence intensity for the control sample (X-ray irradiated water solutions without nanoceria). Concentration of coumarin in all samples was equal to 10^{-4} mol/l. X-ray tube ($U = 30$ kV, $I = 20$ mA) was positioned at $d = 30$ cm from the cuvette with irradiated solution. All samples were irradiated by X-ray during 30 min.

Concentrations of nanoceria in aqueous solutions were the similar in all experiments and equal to 0.5 g/l.

3. Results and discussion

3.1 Influence of Y^{3+} - and Zr^{4+} -doping on the $\cdot OH$ scavenging ability of nanoceria

Cerium oxide-based materials even before being acclaimed as highly-effective ROS scavengers have found their application as solid electrolytes for solid oxide fuel cells (SOFCs) and oxygen storage components in automotive exhaust emission control systems. Mixed ceria-yttria ($CeO_2-Y_2O_3$) solid electrolytes have demonstrated high efficiency for SOFCs operating at relatively low temperatures (600–700°C) [11]. Similarly, ceria-zirconia (CeO_2-ZrO_2) is widely used nowadays in automotive exhaust emission control systems [12]. Overall, the studies have shown that the oxygen storage capacity (OSC) of ceria-based materials which is determined by the content of oxygen vacancies and their mobility could be sufficiently improved by ceria doping with Y^{3+} - or Zr^{4+} ions. While incorporation of Y^{3+} ions leads to formation of additional oxygen vacancies required for charge compensation, doping by Zr^{4+} (which are sufficiently smaller than Ce^{4+} ones) decreases the formation energy of oxygen vacancies due to distortion of the crystal lattice and loosening of the oxygen bonds [13]. In the same way, Y^{3+} - and Zr^{4+} -doping should increase the number of Ce^{3+} ions in the ceria structure as the additional oxygen vacancies take part in formation of $Ce^{3+}-O_v-Ce^{3+}$ (in ceria-zirconia), and $Ce^{3+}-O_v-Y^{3+}$ (in ceria-yttria) complexes.

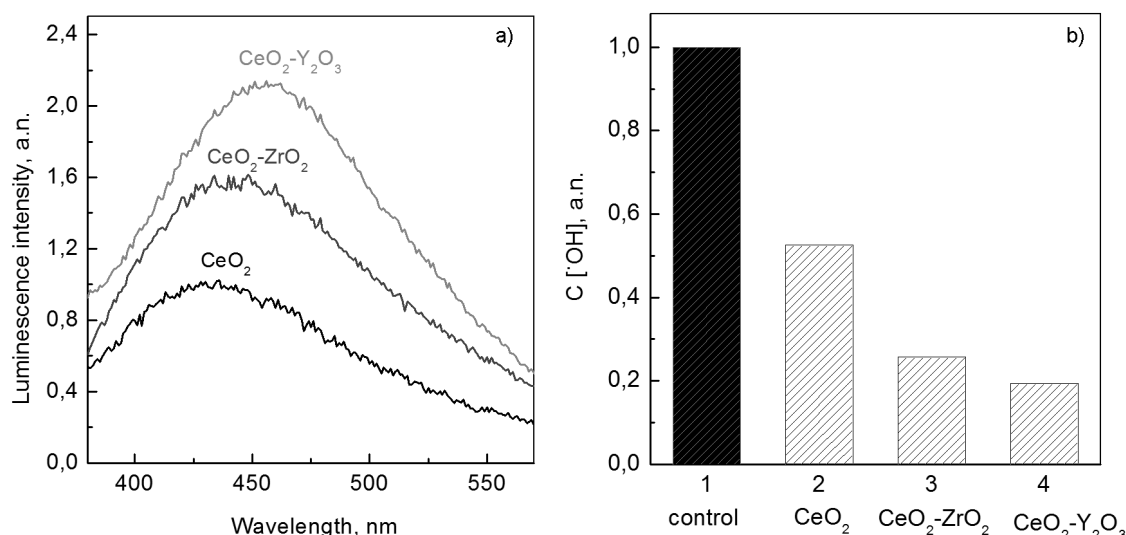


Fig. 2. a) Luminescence spectra of nanoceria (2 nm), Y³⁺- and Zr⁴⁺-doped nanoceria (2 nm), $\lambda_{exc} = 325$ nm; b) Concentration of ·OH radicals in X-ray irradiated water solutions with nanoceria (2 nm), Y³⁺- and Zr⁴⁺-doped nanoceria (2 nm) determined by coumarin test. Control sample — water solutions without nanoceria.

Our previous studies have shown that the content of Ce³⁺ ions in nanoceria and its change during interaction with ROS can be easily estimated using luminescence spectra of ceria nanoparticles [14–16]. These spectra consist of a single wide band with maximum at 390–440 nm (depending on the size of nanoparticle) (Fig. 1a). Using the excitation spectra and decay curves of this band, it was attributed to $5d \rightarrow 4f$ luminescence of Ce³⁺ ions. Decrease of the size of ceria nanoparticle leads to increase of Ce³⁺ content that can be seen from the Fig. 1a as an increase of Ce³⁺ luminescence intensity. In the same way, doping by Y³⁺ and Zr⁴⁺ ions as well as high-temperature treatment of nanoparticles in reducing atmosphere increases the intensity of this band. The dependence of Ce³⁺ luminescence band in 50 nm ceria nanoparticles on the concentration of Y³⁺- and Zr⁴⁺ ions was analyzed in our previous paper [17] and can be seen in the Fig. 1b. The intensity of Ce³⁺ luminescence band strongly increases at both Y³⁺- and Zr⁴⁺-doping, for Zr⁴⁺-doping this effect strongly depends on the treatment atmosphere being sufficiently higher for reducing atmosphere.

In 2 nm nanoceria the content of Ce³⁺ ions is sufficient even without doping or treatment in reducing atmosphere (Fig. 1a). So, the effect of Y³⁺- and Zr⁴⁺-doping ($C = 10$ at. %) on the Ce³⁺ luminescence intensity is less pronounced than for 50 nm

nanoparticles but still sizeable (about 2.1 times for CeO₂-Y₂O₃ and 1.6 times for CeO₂-ZrO₂) (Fig. 2a). The luminescence bands for mixed nanocrystals are slightly red-shifted having the maxima at 445–450 nm as compared to 440 nm for 2 nm nanoceria. The nanoparticles were tested for ·OH scavenging ability using the coumarin test. Coumarin is non-fluorescent but it turns to fluorescent 7-hydroxycoumarin (7OHC) (umbelliferone) by reaction with hydroxyl radicals. The hydroxyl radicals were generated using X-ray ($U = 30$ kV, $I = 20$ mA) and the fluorescence of 7-hydroxycoumarin was used for monitoring ·OH scavenging action of NPs. As was expected, the ·OH scavenging activity of NP correlates with Ce³⁺ content being the highest for Y³⁺-doped nanoceria (about 2.7 times higher than for undoped nanoceria); for Zr⁴⁺-doped nanoceria the ·OH scavenging activity was about 2 times higher than for undoped nanoceria. These results clearly confirm that doping of nanoceria leads to improving its ·OH scavenging properties, as well as the role of increase of Ce³⁺ content in the observed effect.

3.2 Influence of pre-irradiation on the ·OH scavenging ability of nanoceria

The role of UV irradiation in nanoceria antioxidant performance was discussed in our previous papers. Both irradiation of nanoceria solutions before ROS addition (pre-irradiation) [18] and irradiation of nanoceria solutions after ROS addition [19] speed up the process of ROS elimination. In

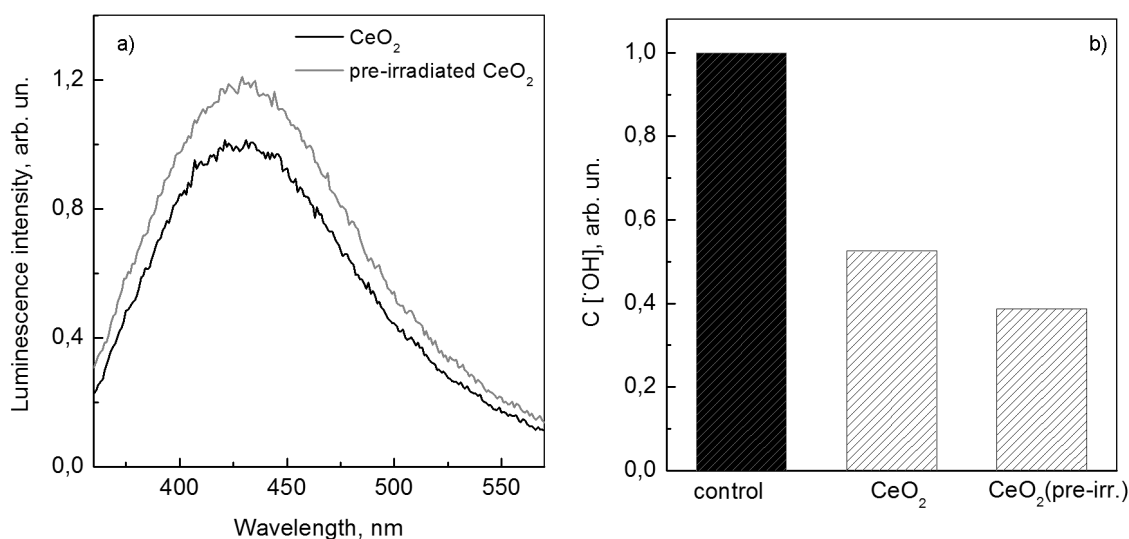


Fig. 3. a) Luminescence spectra of nanoceria (2 nm) with and without laser pre-irradiation (1.5 h, $\lambda = 325$ nm); b) Concentration of $\cdot\text{OH}$ radicals in X-ray irradiated water solutions with nanoceria (2 nm) subjected to laser pre-irradiation. Control sample — water solutions without nanoceria.

[18] was shown that UV pre-irradiation of 2 nm nanoceria leads to change of $\text{Ce}^{3+}/\text{Ce}^{4+}$ ratio leading, in turn, to improving its antioxidant properties which were estimated by nanoceria-induced suppression of formation of diene conjugates at the autooxidation of egg lecithin. However the test used in [18] was not selective for specific type of ROS. For elucidation of the role of pre-irradiation on the $\cdot\text{OH}$ scavenging properties of nanoceria here we have used the coumarin test. The nanoceria water solutions were irradiated by 325 nm laser during 1.5 h and their luminescent and antioxidant properties were compared with the ones of non-irradiated samples. In Fig. 3a the luminescence spectra of irradiated and non-irradiated nanoceria are shown. Laser irradiation leads to increase of Ce^{3+} luminescence intensity in 1.2 times. The coumarin test has shown increase in $\cdot\text{OH}$ scavenging ability in 1.4 times for irradiated samples as compared to non-irradiated ones. So, independently on the method of changing Ce^{3+} content in nanoceria, the NPs with higher Ce^{3+} content demonstrate better $\cdot\text{OH}$ scavenging properties.

The experimental results clearly indicate that doping nanoceria by Y^{3+} and Zr^{4+} ions as well as pre-irradiation of nanoceria water solutions leads to increase of Ce^{3+} content in nanoceria and to improvement of its $\cdot\text{OH}$ scavenging properties. Increase of Ce^{3+} content in Y^{3+} -doped nanoceria is determined by formation of $\text{Ce}^{3+}-\text{O}_v-\text{Y}^{3+}$ complexes.

The number of such complexes at low Y^{3+} concentration (10 % and less) should be proportional to yttrium concentration, while at higher Y^{3+} content $\text{Y}^{3+}-\text{O}_v-\text{Y}^{3+}$ complexes should be dominant. For Zr^{4+} -doped nanoceria the increase of Ce^{3+} concentration stems from increase of the content of oxygen vacancies due to decrease of formation energy of oxygen vacancies near Zr^{4+} ion incorporated to ceria lattice leading to formation of additional $\text{Ce}^{3+}-\text{O}_v-\text{Ce}^{3+}$ complexes. Pre-irradiation increases the number of $\text{Ce}^{3+}-\text{O}_v-\text{Ce}^{3+}$ complexes as well. According to [20] the energy of oxygen vacancy formation on the (100) ceria surface is about 3 eV, so 325 nm (3.8 eV) laser irradiation can lead to formation of additional oxygen vacancies. Moreover, according to [21] the energy of oxygen vacancy formation decreased sufficiently if two electrons remained after oxygen desorption from the surface are localized on nearby Ce^{4+} ions (i.e. if $\text{Ce}^{3+}-\text{O}_v-\text{Ce}^{3+}$ complexes are formed). So, continuous laser irradiation of nanoceria water solution increases the oxygen deficiency of NPs and provides additional $\text{Ce}^{3+}-\text{O}_v-\text{Ce}^{3+}$ complexes which can be used for $\cdot\text{OH}$ scavenging.

The mechanism of $\cdot\text{OH}$ scavenging by nanoceria was proposed in [9] and consists in oxidation of Ce^{3+} ions by $\cdot\text{OH}$ by the following route: $\cdot\text{OH} + \text{Ce}^{3+} \rightarrow \text{OH}^- + \text{Ce}^{4+}$. This process is accompanied by the change of oxygen stoichiometry of nanoceria as whole. The recovery of initial content of

Ce^{3+} ions ($\text{Ce}^{4+} \rightarrow \text{Ce}^{3+}$) according to [9] and our own findings for other ROS types [16, 22] is accompanied by reverse change of oxygen stoichiometry i.e. by oxygen removal from ceria nanoparticle. The content of Ce^{3+} ions after recovery is determined by equilibrium content of oxygen vacancies in nanoceria for specific size and concentration of doped ions, so it should be the same as before interaction with $\cdot\text{OH}$.

So, higher initial content of Ce^{3+} ions provide ceria NPs by more pronounced $\cdot\text{OH}$ scavenging ability. This ability is based on the $\text{Ce}^{3+} \rightarrow \text{Ce}^{4+}$ reversible switching accompanied by oxygen accumulation (during $\cdot\text{OH}$ -nanoceria interaction) and release (on the stage of recovery of initial Ce^{3+} content). Both Y^{3+} - and Zr^{4+} -doping, and pre-irradiation increases the content of oxygen vacancies in nanoceria, and, so the number of Ce^{3+} ions improving the $\cdot\text{OH}$ scavenging properties of nanoceria.

4. Conclusions

The influence of Y^{3+} - and Zr^{4+} -doping, and pre-irradiation on the $\cdot\text{OH}$ scavenging properties of nanoceria was studied using spectroscopic techniques. The dependence of $\cdot\text{OH}$ scavenging properties on the content of Ce^{3+} ions in nanoceria was confirmed by the simultaneous study of luminescence spectra and nanoceria — $\cdot\text{OH}$ interaction processes. Incorporation of doped ions as well as 325 nm laser pre-irradiation of nanoceria water solutions led to sufficient increase of the content of Ce^{3+} ions accompanied by corresponding improvement of $\cdot\text{OH}$ scavenging characteristics. While Y^{3+} -doping provided increase of Ce^{3+} content due to formation of $\text{Ce}^{3+}-\text{O}_v-\text{Y}^{3+}$ complexes, Zr^{4+} -doping and pre-irradiation led to formation of additional oxygen vacancies and $\text{Ce}^{3+}-\text{O}_v-\text{Ce}^{3+}$ complexes by lowering the energy required for oxygen release from nanoceria lattice. Overall, remarkable $\cdot\text{OH}$ scavenging properties and reversible redox characteristics make Y^{3+} - and Zr^{4+} -doped and pre-irradiated ceria nanocrystals the potent materials for $\cdot\text{OH}$ scavenging in living cells.

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