Induction and inhibition of free radicals by the $GdVO_4:Eu^{3+}$ and CeO_2 nanoparticles under X-ray irradiation

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The effect of aqueous colloidal solutions of $GdVO_4:Eu^{3+}$ and CeO_2 nanoparticles on the efficiency of generation or inhibition of free radicals under X-ray irradiation was studied. It is shown that gadolinium orthovanadate acts as a free radical generator, and cerium dioxide as an inhibitor.

Keywords: orthovanadate, cerium dioxide, nanoparticles, X-ray irradiation, free radicals.

Проведены исследования влияния водных коллоидных растворов наночастиц $GdVO_4:Eu^{3+}$ и CeO_2 на эффективность генерации или ингибирования свободных радикалов при рентгеновском облучении. Показано, что ортованадат гадолиния выступает в качестве генератора свободных радикалов, а диоксид церия — в качестве ингибитора.

Індукування та інгібування вільних радикалів наночастинками $GdVO_4:Eu^{3+}$ і CeO_2 при рентгенівському опроміненні. *В.К.Клочков, О.О.Сєдих, Г.В.Григорова, О.Г.Вягін, О.Д.Ополонін, Ю.В.Малюкін*

Проведено дослідження впливу водних колоїдних розчинів наночастинок $GdVO_4:Eu^{3+}$ і CeO_2 на ефективність генерації або інгібування вільних радикалів при рентгенівському опроміненні. Показано, що ортованадат гадолінію виступає як генератора вільних радикалів, а діоксид церію — як інгібітор.

1. Introduction

Despite the fact that there have been significant developments in anti-cancer technology, such as radiotherapy, chemotherapy and hormone therapy, cancer still remains as the second leading cause of death following heart disease in the world. In addition to the use of nanoparticles (NPs) in medicine, which has successfully improved the specificity of modern chemotherapeutics in recent years [1–3], a combination of radiotherapy with nanoparticles has been proposed as a new alternative to improve protocols of treatment [4–7]. One of the problems of radiotherapy is that its effectiveness is limited; on the one hand, by the radiosensitivity of the tissues surrounding the tumor, on the other hand, by the radioresistance of the malignant neoplasm itself. The application of NPs as radiosensitizers is a promising strategy to increase the efficiency of radiotherapy. The concept is to increase absorption capacity of the tumor through the incorporation of NPs and thus to achieve radiosensitisation and ion beam radiation to achieve a higher, more localized dose. Consequently, the differential effect between normal and tumor tissues could be improved [8].

Among all radiosensitizing nanomaterials, carbon nanotubes [9, 10], gold [11–13] and other metallic nanoparticles [14] can be mentioned. Recent studies have also reported the use of lanthanide-based NPs, ti-

tanium oxide and iron oxide nanoparticles or cadmium selenide quantum dots [15–18]. Mechanism of induced radiosensitivity by most of these sensitizers consists in ROS (reactive oxygen species) production such as superoxide ions, hydrogen peroxide, hydroxyl radicals and singlet oxygen ($^1O_2$). Increase of the concentration of free radicals activates necrosis and apoptosis reactions and finally leads to cell death [19, 20]. At the same time, it should be noted that nanoparticles-enhanced radiation sensitivity depends on the type and size of nanoparticles, cell line, irradiation energy, concentration and intracellular localization.

Another type of nanoparticles that attract attention of researchers due to their biological activity is nanoparticles based on orthovanadates of rare-earth elements. These particles possess antioxidant properties [21], the ability to accumulate selectively in individual cells and cell organelles [22], the ability to suppress the growth of malignant tumors [23, 24], as well as radioprotective and adaptogenic properties [25].

Previously, we have demonstrated that at UV irradiation of hydrosols containing rare-earth orthovanadate NPs induce formation of radicals in water [26]. Also, for orthovanadate NPs with different sizes it was shown that intensity of the photocatalytic process increases with increase of the linear sizes of NPs. For determination of the intensity of radical formation in the nanoparticles colloidal system, the organic compounds able to interact with radicals with formation of the products of destruction were used. Usually, for these purposes dyes easily destructing under an action of free radicals are chosen. During interaction with the free radicals decrease of an optical density of characteristic dye bands in the absorption spectra is observed [26].

In the mentioned paper we have also shown that, in contrast to orthovanadates, nanocrystals of cerium dioxide CeO_2 under UV irradiation inhibit formation of radicals in water and play a role of a radical sponge. In this case, the optical density of the dye in the presence of CeO_2 nanoparticles after UV irradiation practically does not change.

The task of this paper was to find out whether the water colloidal solutions of the orthovanadates and cerium dioxide NPs exhibit the same properties with respect to induction and inhibition of free radicals under X-ray irradiation as for ultraviolet irradiation.
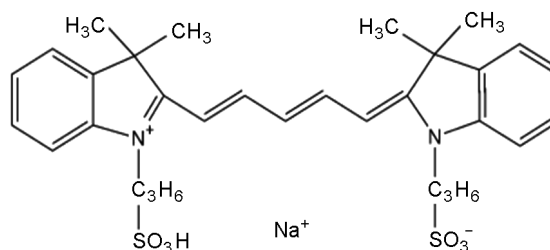

Fig. 1. Structural formula of 1,1'-di(3-sulfopropyl)-3,3,3',3'-tetramethylindodicarbocyanine sodium salt (DiD–C3S).

2. Experimental

Materials. Lanthanide chlorides ($GdCl_3$ and $CeCl_3$, 99.9 %) and anhydrous sodium metavanadate ($NaVO_3$, 96 %) (all firms "Acros organics") were all used without further purification. Sodium citrate ($Na_3C_6H_5O_7$, 99 %), hexamethylenetetramine (($CH_2)_6N_4$, 99 %), hydrogen peroxide (H_2O_2, 35 %), ammonium hydroxide (NH_4OH, 25 %) from "Macrochem" Co. Ltd. were used. Na_3VO_4 solution with pH value of 13 was obtained by adding solution NaOH (1 mol/L) to $NaVO_3$ water solution. Doubly distilled water was used throughout this study. Anionic dye 1,1'di(3-sulfopropyl)-3,3,3',3' tetramethylindodicarbocyanine sodium salt (DiD–C3S) was synthesized in the Institute for Scintillation Materials NAS of Ukraine. For reference, the structure of DiD–C3S is shown below (Fig. 1).

Stable colloidal solutions of elongated $GdVO_4$:Eu^{3+} NPs and spherical CeO_2 NPs were prepared by the method described earlier using sodium citrate as a stabilizing agent [27, 28]. The solution is transparent in transmitted light and opalescent in lateral light (Tyndall cone). Colloidal particles pass easily through nitrocellulose ultrafilters with a pore diameter of 100 nm. The solid phase concentration is 1 g/L. pH is 7.4–7.6. The solutions were stored in sealed ampules without changing their properties for more than 2 months at normal conditions. The inorganic NPs carry a negative charge with the average value of ζ-potential ranging from −22 to −30 mV. The ζ-potential depends on a storage period.

All types of NPs had a negative surface charge, so, to exclude an electrostatic interaction with NPs and localization of the dye on the surface of NPs, as an indicator of free radicals DiD–C3S anionic dye was used. At DiD–C3S anionic dye addition to colloi-
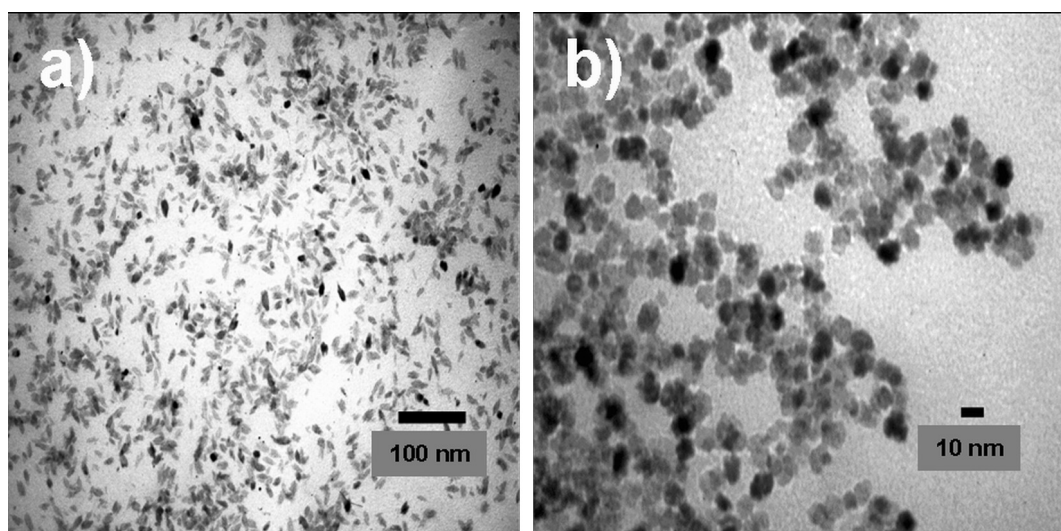
Fig. 2. TEM images of nanoparticles in colloidal solutions. Spindle-like $GdVO_4{:}Eu^{3+}$ NPs — a); spherical CeO_2 NPs — b).

dal solutions of NPs of all types no changes in dye absorption spectrum were observed. Any changes of ζ-potential of NPs, increase of hydrodynamic diameter of NPs or coagulation also were not registered confirming an absence of interaction of the anionic dye with the surface of negatively charged NPs. Besides, DiD–C3S is characterized by easy destruction in the presence of active radicals.

Procedure and analysis. Particle morphology and size distribution were determined by TEM. Specimens were prepared by placing a drop of colloidal solution onto a 200-mesh holey carbon copper grid. The grid was air-dried under dust-free conditions and examined in TEM 125K (Selmi) electron microscope at 100 kV.

Irradiation experiments were carried out as following: the hydrosol investigated was poured into a quartz cuvette with an optical path length of 1.0 cm, after that DiD–C3S dye solution was added. The hydrosol with the dye added was stirred and then the cuvette was irradiated from above (from the open part) by an X-ray using ISOVOLT 160 Titan E apparatus with a tungsten cathode for 30 min. The voltage on the tube varied from 30 kV and 120 kV. The distance from the X-ray tube to the irradiated samples was 50 cm. The concentration of nanoparticles in aqueous solutions of NPs varied from 0.01 to 1 g/L. The concentration of the dye was the same in all the samples and was $6.5{\cdot}10^{-6}$ M.

The effect of nanoparticles on the destruction of the dye due to interaction with free radicals was determined from the change in the optical density of the dye in the samples. Absorption spectra were recorded using a Specord 200 spectrophotometer (Analytik Jena, Germany) at $\lambda_{max} = 642$ nm. The control sample was an aqueous solution of the dye DiD–C3S. Temperature inside the sample compartment of the spectrophotometer was $25{\pm}0.25°$C.

3. Results and discussion

In this paper, we present the results of an investigation for nanoparticles of two types: orthovanadates $GdVO_4{:}Eu^{3+}$ with size of 8×25 nm and spherical nanoparticles of cerium dioxide CeO_2 with diameter of 10 nm, TEM image of NPs is shown in Fig. 2.

Under UV irradiation, these nanoparticles exhibit photocatalytic activity; in particular, orthovanadate NP acts as a free radical generator, and cerium dioxide as an inhibitor [26]. Unlike UV irradiation which is directly absorbed by nanoparticles, the ionizing radiation leads to formation of free radicals mainly by radiolysis of the solvent (in this case, water). For example, in a number of papers [4, 29] it was shown that despite the fact that inorganic nanoparticles usually have a much larger mass attenuation coefficient of X-ray radiation than water, for commonly used concentrations of nanoparticles in solutions (units and tenths g/L), their mass fraction with respect to water is rather small, and, as a result, most of the primary incident ionizing radiation is absorbed in water (usually, tens of percent for water and one or less percent for nanoparticles depending on the energy). Nevertheless, in the same studies it was
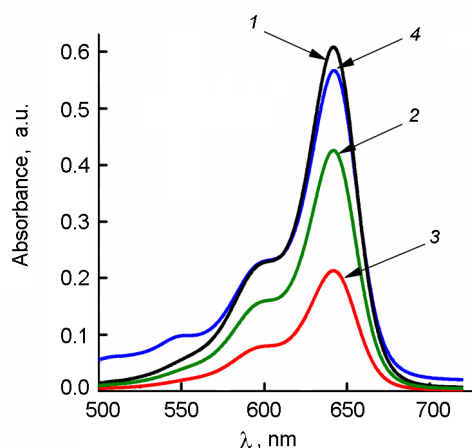
Fig. 3. Degradation of DiD–C3S dye at X-ray irradiation during 30 min. Control sample before irradiation — *1*, after irradiation: control sample — *2*, in presence of $GdVO_4{:}Eu^{3+}$3 NPs — *3*, in presence of CeO_2 NPs — *4*.

noted that for nanoparticles, the total dose of absorbed ionizing radiation increases, most likely, due to a local increase of the absorbed dose in the immediate vicinity of the nanoparticles.

During the radiolysis in the water, various radiolytic products with different lifetimes and reactivity are formed ($OH^{\cdot}$, H_2O_2, $O_2^{\cdot}$ etc.) [5, 26]. As a rule, these products cause a change in the composition and structure of dye molecules as a result of the interaction of the latter with oxygen-containing radicals and ion radicals leading, for example, to discoloration of dyes [26]. It should be noted that this paper does not consider any specific mechanisms of interaction of radiolysis products with dye or nanoparticles, and only the influence of nanoparticles on the degree of discolouration of the dye under the influence of X-rays are described.

At X-ray irradiation (at tube voltages 30 kV) of the solutions of cerium dioxide and orthovanadate NPs (0.2 g/L) with DiD–C3S indicator during 30 min decrease of the characteristic indicator absorption band is observed which can be ascribed to dye degradation (Fig. 3). In the presence of orthovanadate NPs total bleaching of the solution is observed, while presence of cerium dioxide NPs leads only to partial dye degradation. Also destruction of the dye after irradiation of the solution with cerium dioxide NPs is less than in the control sample.

In Fig. 4 the histogram of the change of optical density of the dye in the presence of
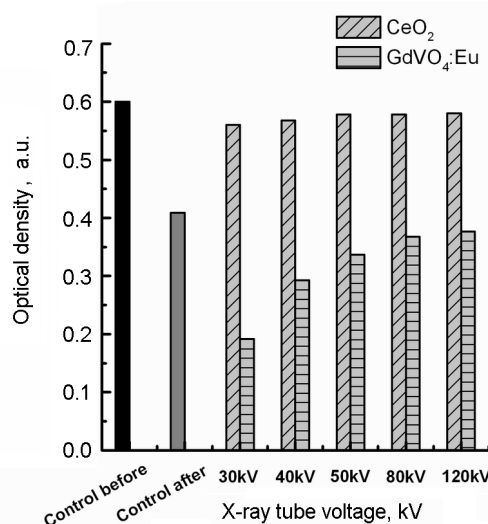

Fig. 4. The change of optical density of the dye DiD–C3S in the presence of nanoparticles after X-ray irradiation at different tube voltage in comparison with the control sample (DiD–C3S dye) before and after irradiation.

$GdVO_4{:}Eu^{3+}$ and CeO_2 nanoparticles, as well as of the control sample (dye solution without nanoparticles), irradiated with X-ray is shown. It can be seen that for the control sample, the optical density after irradiation of 30 kV decreases from 0.6 to 0.4, i.e. by about 34 %. In the presence of $GdVO_4{:}Eu^{3+}$ nanoparticles, the optical density of the samples decreases more than in the control — up to 0.2 (at 30 kV). This fact confirms that orthovanadates act as an effective generator of free (active) radicals. Generation of free radicals by orthovanadates can be a consequence of the interaction of nanoparticles both directly with falling X-ray quanta and with secondary particles, for example, delta electrons, Auger electrons etc formed in the bulk of the sample after the interaction of ionizing radiation with water.

In this case, the dependence of the decrease in the optical density on the voltage on the X-ray tube (i.e., on the effective energy of the incident radiation) at which irradiation was carried out is also observed. As can be seen from Fig. 4, the strongest decrease in optical density occurs at the lowest voltage on the tube (30 kV), and the least strong at the highest voltage (120 kV). Most likely, this is due to decrease in the efficiency of absorption of X-ray quanta of higher energies both in water and directly in nanoparticles.

As seen in Fig. 4, in the presence of CeO_2 nanoparticles, the optical density of
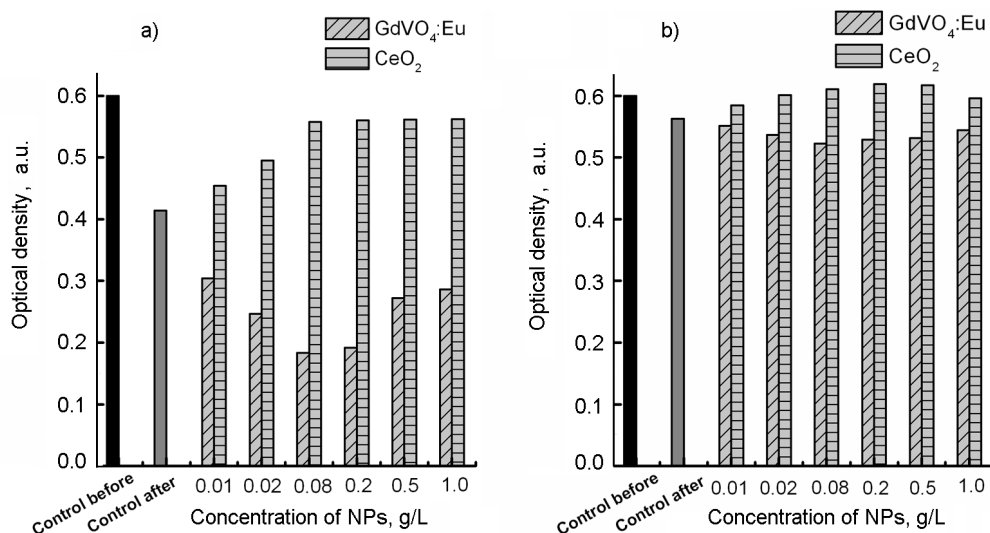
Fig. 5. The change of optical density of the dye DiD–C3S in the presence of different concentration of nanoparticles after irradiation with X-ray at 30 (a) and 120 (b) kV voltage on the tube in comparison with the control sample (DiD–C3S dye) before and after irradiation.

the dye decreases by less than 10 % and weakly depends on the voltage on the tube, which indicates the high efficiency of inhibition of free radicals arising during radiolysis of water by CeO_2 nanoparticles.

We also studied the dependence of the intensity of generation and inhibition of radicals in solutions on the concentration of nanoparticles. In Fig. 5 the histograms of the change in the optical density of the samples as a function of the concentration of nanoparticles at voltages on the tube of 30 kV (a) and 120 kV (b) are shown. The dependences for both NPs are sufficiently monotonic, with a minimum or maximum at the concentrations most effective for given conditions. It is evident that for orthovanadates the most effective concentrations (the lowest optical density) under the selected experimental conditions are 0.08 g/L and 0.2 g/L for 30 kV and 120 kV, respectively. If we compare the optical densities of the control samples after irradiation of 30 kV and 120 kV, it can be seen that in the case of 120 kV, the optical density decreases not as much as at 30 kV, to 0.56 and to 0.42, respectively (Fig. 5 a,b). Also, at 120 kV, the decrease in the optical density of the dye in solutions with orthovanadates is not as strong as at 30 kV. So, the efficiency of absorption of X-ray quanta of higher energies in the sample volume decreases and, consequently, the amount of radiolysis products generated in water is lower, as well as the intensity of generation of free radicals by orthovanadate nanoparticles.

For CeO_2 nanoparticles demonstrating the opposite effect of inhibition of ROS, a monotonous increase in optical density is observed at 30 kV with increasing concentration reaching finally the plateau at 0.08 g/L. At 120 kV optical density of the dye varies slightly with an increase in the concentration of NPs of cerium oxide, while in both cases the optical density of the dye in the presence of CeO_2 NPs is greater than in the control samples without NPs. It should be noted that the obtained concentration dependences for NPs, although they are in accordance with the laws of absorption of ionizing radiation, reflect only the specific experimental conditions and their character under other conditions may differ.

These results, without the use of additional calculations and/or direct experiments on X-ray absorption by colloidal solutions, do not allow obtaining any quantitative estimates with respect to the generation or inhibition of active radicals by nanoparticles. Nevertheless, an important practical result is that $GdVO_4$:Eu^{3+} and CeO_2 NPs under irradiation with ionizing radiation qualitatively manifest themselves in the same way as in the case of UV excitation into the absorption band of nanoparticles — orthovanadates act as generators, and CeO_2, on the contrary, as inhibitors of ROS. The mechanisms of action of nanoparticles at UV and X-ray irradiation may differ, but their final result will be either the formation of additional free radicals (in the presence of $GdVO_4$:Eu^{3+} NPs) or their inhibition (in the presence of CeO_2 NPs).

4. Conclusions

In this paper it was shown that the nanoparticles of orthovanadates and cerium dioxide irradiated with X-ray ionizing radiation exhibit the same properties with respect to the generation ($GdVO_4$:Eu^{3+}) or inhibition (CeO_2) of free radicals, as at the direct excitation into ultraviolet absorption bands of these nanoparticles. The obtained results indicate the prospects of using the orthovanadate nanoparticles in the biomedical field, in particular, in the radio-dynamic therapy of oncological diseases.

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