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UV-shielding property, photocatalytic activity and photocytotoxicity of ceria colloid solutions

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ABSTRACT

UV-shielding property, photocatalytic activity and cytotoxicity (including photocytotoxicity) of citrate-stabilized ceria colloid solutions were studied. It was established that UV-shielding property (namely, the sun protection factor, the critical absorption wavelength and the *UVA/UVB-ratio*) of ceria nanoparticles are as good as those of titanium dioxide and zinc oxide nanoparticles. It was further demonstrated that ceria nanoparticles possesses substantially lower photocatalytic activity, which additionally decreases upon decrease in ceria particle size. It was found that colloid ceria solutions are non-toxic to mouse fibroblasts (L929) and fibroblast-like cells of African Green monkey (VERO). Moreover, ceria nanoparticles are capable to protect these cells from UV-irradiation-induced damage. It was proposed that nanocrystalline ceria could be used not only as UV-blocking material, but also as prophylactic and even therapeutic compound for sunburns treatment.

1. Introduction

Nanocrystalline semiconductor materials are widely used in sunscreen cosmetics. Nowadays, most of inorganic UV-blocking filters are based on titanium dioxide (TiO_2) and zinc oxide (ZnO). However, a number of reports exist indicating the possibility of brain cells [1], blood lymphocytes [2] and lymphoblastic cells [3] damage by titania nanoparticles. Moreover, nanoparticles of zinc and titanium oxides possess enormous photocatalytic activity [4,5], thus inducing the increase in their toxicity upon irradiation. Reactive oxygen species (ROS) forming during photocatalytic processes decompose not only components of cosmetics but even skin cells [6,7]. It was demonstrated that under UV-irradiation TiO_2 and ZnO nanoparticles being the part of sunscreen cosmetics generate hydroxyl radicals [8–10,3] damaging DNA of skin cells [11]. Photocytotoxicity of titania against fibroblasts has been also confirmed [12].

Cerium dioxide (CeO_2) is one of the today's most promising nanobiomaterials [13–16]. Ceria becomes strongly non-stoichiometric in nanocrystalline state and due to this reason is able to participate in various redox processes [17,18], in particular, to inactivate some of the most toxic ROS, such as superoxide radical [13], hydrogen peroxide [14] and nitroxyl radical [19]. Due to the high concentration of oxygen defects in ceria lattice the recombination of free charge carriers (electrons and holes) forming upon UV-irradiation of ceria proceeds very rapidly. It should be also noted that UV-extinction coefficient of ceria is rather high; therefore this compound is considered as a promising UV-filter in sunscreen cosmetics [20–22]. Moreover, vacancy engineered ceria nanostructures can protect from radiation-induced cellular damage [23], radiation-induced pneumonitis [24] and can prevent retinal degeneration by photons of light [25].

Thus the aim of this work consists of thorough investigation of the possibility of nanocrystalline ceria use in sunscreen cosmetics, including study of its sunshielding properties, photocatalytic activity and cytotoxicity upon UV-irradiation.

2. Experimental

Colloid solutions of ceria nanoparticles (0.1 M) stabilized by sodium citrate were synthesized according to [26,27]. Nanoparticles obtained were of ultra small size ($\leqslant$2 nm), stable in water, various buffer solutions, and biological fluids in a wide range of pH values (from 4 to 12). Our previous studies indicate also that such nanoparticles can participate in redox processes in aqueous media [27].

Ceria nanopowders of different particle size used hereafter for comparative studies of photocatalytic activity were synthesized

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by rapid mixing of aqueous-isopropanolic (1/1 vol/vol) solution of cerium(III) nitrate (0.08 M) and aqueous ammonia solution (3 M) followed by annealing of precipitates in a muffle furnace at 60, 300, 400 and 600 °C for 2 h.

Powder X-ray diffraction (XRD) analysis of ceria nanopowders was carried out on a Rigaku D/MAX 2500 diffractometer (Cu Kα radiation, instrumental broadening 0.10 ± 0.01 2θ). Particle size (D) of nanocrystalline ceria was calculated using Scherrer formula where coefficient of anisotropy was set to one. Line profiles for (1 1 1) and (2 0 0) reflections were fitted to pseudo-Voigt functions. D values calculated from full width at half maximum (FWHM) of (1 1 1) and (2 0 0) diffraction lines differed by no more than 0.2 nm for all the samples. To determine the values of unit cell parameter the Rietveld refinement was performed. Peak profiles were fitted to pseudo-Voigt functions in the range 15–90° 2θ taking into account the non-monochromaticity of Cu Kα radiation. Background lines were fitted to 15-order Chebyshev polynomials.

The specific surface area of the CeO_2 nanopowders was determined by low-temperature nitrogen adsorption measurements with a QuantaChrome Nova 4200B analyzer. The samples were outgassed at 40 °C in vacuum for 5 h. The sample surface was analyzed by a multipoint Brunauer–Emmett–Teller (BET) method.

Photocatalytic activity of ceria and titania (Degussa P25) was studied in a model reaction of methyl orange azo dye photodegradation. Colloid solutions and suspensions of ceria and titania (3 g/l) were thermostated at 36 °C for 12 h. Then aliquots of the solutions (2 ml) were placed into microreactor thermostated at 36.6 °C (normal body temperature) and mixed with the aqueous solution of methyl orange (13 mg/l). Solutions obtained were additionally mixed for 2 h to reach adsorption/desorption equilibrium. Then the reactor was irradiated by a xenon lamp (OceanOptics HPS-2000) for 60 min. The concentration of the dye was monitored directly in course of the photocatalytic reaction by use of OceanOptics QE-65000 spectrometer. Spectra were recorded every 30 s. Resulting dependencies of dye concentration on the duration of UV-irradiation were used to determine methyl orange photodegradation reaction constants which were used further as a measure of photocatalytic activity of ceria and titania samples.

Toxicity of nanocrystalline ceria sols under UV-irradiation and in the dark was studied using reference cell lines including mouse fibroblasts (L929) and fibroblast-like cells of African Green monkey (VERO) from cell culture collection of Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of Ukrainian National Academy of Sciences.

To form cell monolayers, the aliquots (0.1 ml) of suspension containing 5×10^5 cell/ml were placed in 96-well Costar plates or on coverslips placed in 6-well plates and incubated at 37 °C for 24 h in a TC-80 M-2 thermostat in humid air (98%) containing 5% CO_2. Synthetic nutrient 199 medium (Biotestlaboratory, Ukraine) supplemented with 5–10% fetal bovine serum (Sigma, USA), 25 mM HEPES, 10 mM glutamine, 10 mM penicillin, and 10 mM streptomycin (100 units/ml of each) was used as the growth medium. Cell supporting medium consisted of nutrient 199 medium, 2% fetal bovine serum, 25 mM HEPES, 10 mM glutamine, 10 mM penicillin, and 10 mM streptomycin (100 units/ml of each). The cell monolayers were washed with 199 medium without fetal bovine serum.

Protective action of ceria nanoparticles was determined by the analysis of L929 and VERO cells viability. Each of the tests was performed in triplicate. Ceria sols of different concentrations (0.16–5.00 mM) were added to the cell monolayers 30 min before, 5 min before or 30 min after UV-irradiation. Dose of UV-irradiation was 30 J/m^2 which corresponds to ~1 TLV (Threshold Limit Value for Ultraviolet Radiation). Viability of the cells was estimated 24 h after single-dose UV-irradiation by measuring optical absorption of supravital dye Neutral Red (NR) absorbed by cells in accordance with the method [28]. NR is a weak cationic dye which can easily penetrate cell membranes as well as lysosomes by non-ionic diffusion. Any changes in cell surface state or in susceptibility of lysosomal membranes are accompanied by lysosomal fragility increase and by the other irreversible intercellular changes. Such changes lead to decrease in absorption of NR dye. The method described allows distinguishing between viable, injured or dead cells. It is very sensitive and delivers comprehensive information on the cell integrity and the cell growth braking. The positive control consisted of staining intact cells, the negative control consisted of staining UV-irradiated cells. Optical density of cells stained with NR dye was measured using a vertical beam Labsystem Multiscan Spectrometer at 540 nm. Percentage of cells absorbing NR was determined as the ratio of the difference in optical density of the stained intact cell and the stained test cells to the optical density of stained intact cells:

$$(A_{control} - A_{test})/A_{control} \times 100. \quad (1)$$

Here, $A_{control}$ is the optical density of the stained intact cells, A_{test} is the optical density of the stained test cells.

Cytotoxicity criteria IC50 and IC100 corresponded to the concentration of ceria sols at which the level of NR dye absorption by the cells decreased by 50% and 100%, respectively.

Statistical treatment of data obtained was performed using a BioStat 2009 Professional 5.8.1 software in accordance with recommendations [29,30]. Arithmetic mean (M) and standard deviation (m) values were thus found. Validity check for the null hypothesis was performed using a nonparametric Wilcoxon–Mann–Whitney (WMW, U) and a Kolmogorov–Smirnov (KS) criteria. The difference between groups was judged to be statistically significant at $p < 0.05$.

Additional method for cells viability and integrity visualization consisted in staining cell monolayers by mixture of luminescent dyes Hoechst 33342 (HOE) and propidium iodide (PI) according to protocol adopted from [31]. A 25 μM working solution of HOE was prepared by 1:4 dilution of 0.1 mM HOE aqueous stock solution with a phosphate buffer solution (PBS). In turn, concentration of working solution of PI in PBS was 20 μg/ml. Before staining the cells grown on coverslips were washed in Dulbecco's PBS. The samples were kept at 4 °C throughout the washing and staining procedure to minimize changes in the living/dead cell ratio. 0.1 ml of PI working solution was added to the cells monolayers and then the cells were incubated for 30 min on ice. Next, 1.9 ml of 25% methanol solution in PBS was added to the cells. Fifty microlitre of HOE working solution were added, the resulting solution was mixed, and the cells were incubated in the dark at 4 °C for 24–72 h before being analyzed. Cells investigation was performed using MBI-15 microscope (LOMO, Russia) equipped with a Canon Digital IXUS 9515 camera. The stained cells appearance was investigated under UV illumination (the source of light was DRS-250 mercury lamp). Excitation wavelength maximum was 365 nm.

3. Results and discussion

3.1. UV-shielding property

UV-shielding properties of ceria nanoparticles were studied in comparison with the corresponding properties of TiO_2- and ZnO-containing systems by measuring the light absorption in the middle (UVB) and near (UVA) ultraviolet regions.

To estimate the efficiency of skin protection in UVB region we used the value of Sun Protection Factor (*SPF*) defined by Sayre et al. [32] as: