



Cite this: *RSC Adv.*, 2021, **11**, 35351

New facets of nanozyme activity of ceria: lipo- and phospholipoperoxidase-like behaviour of CeO_2 nanoparticles†

Madina M. Sozarukova, ^a Elena V. Proskurnina, ^b Anton L. Popov, ^{ac} Alexander L. Kalinkin ^d and Vladimir K. Ivanov ^{*ae}

Cerium dioxide nanoparticles have a special place among engineered nanomaterials due to the wide range of their enzyme-like activities. They possess SOD-, catalase- and peroxidase-like properties, as well as recently discovered phosphatase-, photolyase-, phospholipase- and nuclease-like properties. Advancing biomedical applications of CeO_2 -based nanozymes requires an understanding of the features and mechanisms of the redox activity of CeO_2 nanoparticles when entering the vascular bed, especially when interacting with lipid-protein supramolecular complexes (biomembranes and lipoproteins). In this paper, CeO_2 nanoparticles are shown to possess two further types of nanozyme activity, namely lipo- and phospholipoperoxidase-like activities. Compared to a strong blood prooxidant, hemoglobin, CeO_2 nanoparticles act as a mild oxidising agent, since they exhibit a 10^6 times lower, and 20 times lower, prooxidant capacity towards linoleic acid and phosphatidylcholine hydroperoxides, respectively. Compared to the widespread pharmacological preparation of iron, $\text{Fe}(\text{III})$ carboxymaltose (antianemic preparation Ferinject®), the prooxidant capacity of CeO_2 nanoparticles towards lipid and phospholipid substrates has been shown to be 10^2 times lower, and 4 times higher, respectively. The data obtained on the mechanism of the interaction of nanodisperse CeO_2 with the main components of biological membranes, lipids and phospholipids enable the substantial expansion of the scope of biomedical applications of CeO_2 nanozymes.

Received 7th September 2021

Accepted 22nd October 2021

DOI: 10.1039/d1ra06730c

rsc.li/rsc-advances

Introduction

Several classes of inorganic nanomaterials, including metal oxide and noble metal nanoparticles, exhibit unexpectedly high biological activity due to their ability to mimic some natural enzymes.^{1,2} These materials, known as nanoenzymes or nanozymes, demonstrate relatively high stability and reproducibility of characteristics in wide temperature and pH ranges, as well as biocompatibility, the possibility of additional surface functionalisation and low cost.^{3–7}

Cerium dioxide is a striking representative of a new generation of nanozymes. The enzyme-like properties of nanocrystalline CeO_2 ,

mimicking the functions of some natural enzymes, superoxide dismutase (SOD),^{8–10} catalase¹¹ and oxidase,^{12,13} have recently been supplemented with the discovery of new types of biochemical activity, including phosphatase,¹⁴ photolyase,¹⁵ phospholipase¹⁶ and nuclease-like¹⁷ properties. This makes cerium dioxide a unique multifunctional nanozyme and expands the scope of its future biomedical applications.^{18–21}

Recent studies have demonstrated the role of cerium dioxide as a possible regulator of free radicals in living systems. Nanocrystalline cerium dioxide has dual redox activity, combining pro- and antioxidant properties. The role of CeO_2 as a free radical scavenger has been confirmed by a number of studies demonstrating inhibition of neuronal death in transgenic 5xFAD mice (Alzheimer's disease model),²² reduction of ovarian tumour growth (cancer xenograft model),²³ preventing loss of photoreceptor cell function (P23H-1, the autosomal dominant retinitis pigmentosa model),²⁴ etc.

Despite a number of sophisticated studies, little information is available on the interaction of CeO_2 nanoparticles with biological molecules,²⁵ especially with biomembranes.^{26,27} In eukaryotic cells, phospholipids are the predominant membrane lipids, asymmetrically embedded in a lipoprotein complex.^{28–30} Phospholipids are a class of lipids consisting of a glycerol backbone, a polar group and two hydrophobic acyl chains.²⁸ In

^aKurnakov Institute of General and Inorganic Chemistry, Russian Academy of Sciences, Russian Federation. E-mail: s_madinam@bk.ru; antonpopovleonid@gmail.com; van@igic.ras.ru

^bResearch Centre for Medical Genetics, Russian Federation. E-mail: proskurnina@gmail.com

^cInstitute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Russian Federation

^dMedical Research and Educational Center, Lomonosov Moscow State University, Russian Federation. E-mail: akalinkin@sleelab.ru

^eNational Research University Higher School of Economics, Russian Federation

† Electronic supplementary information (ESI) available. See DOI: 10.1039/d1ra06730c



most eukaryotic membranes, phosphatidylcholine and phosphatidylethanolamine make up about 60–85% of the phospholipid fraction.^{28,31,32} A lipid bilayer is sensitive to free radical oxidation (primary target).^{33,34} Lipid peroxidation (LPO) disrupts cell membrane integrity and plays an important role in the development of cell death mechanisms, including apoptosis, oxytosis and ferroptosis.³⁵ Since the redox activity of the CeO₂ nanzyme assures both pro- and antioxidant properties, studies on cellular and animal models after exposure to CeO₂ nanoparticles have shown either an increase or a decrease in the level of lipid peroxidation products, which are indicators of oxidative stress.^{36–41}

In this study, the chemiluminescence method was used for gathering data on the redox activity of CeO₂ nanoparticles, which has enabled expansion of the list of known types of enzyme-like activities of CeO₂ nanoparticles, to include lipo- and phospholipoperoxidase-like activities. The enzyme-like properties of nanodisperse CeO₂ and the main prooxidant of blood, hemoglobin, as well as the conventional lipid prooxidant, pharmaceutical preparation Ferinject®, which is a colloidal solution of Fe(III) carboxymaltose, were compared to reveal differences in the action mechanism of these prooxidants. The results obtained are of special importance for advancing the pharmacotherapeutic applications of CeO₂ nanoparticles in the treatment of socially significant diseases.

Materials and methods

Synthesis and physicochemical study of citrate-stabilised CeO₂ sol

An unstabilised colloidal solution of CeO₂ nanoparticles (0.13 M) was prepared by thermohydrolysis of an aqueous solution of ammonium cerium(IV) nitrate (#215473, Sigma-Aldrich).⁴² Briefly, an aqueous solution of ammonium cerium(IV) nitrate (100 g L⁻¹) was heated at 95 °C for 24 h. The precipitate formed was washed three times with isopropanol and then redispersed in deionised water and boiled for 1 h, with constant stirring, to remove isopropanol. The concentration of CeO₂ sol was determined gravimetrically. CeO₂ sol was further stabilised by disubstituted ammonium citrate (C₆H₁₄O₇N₂, #247561, Sigma-Aldrich) in a molar ratio of 1 : 1.

X-ray diffraction (XRD) analysis of CeO₂ samples was carried out on a Bruker D8 Advance diffractometer (CuK α radiation, geometry θ –2 θ). The diffraction maxima were identified using the ICDD PDF2 databank. The average hydrodynamic diameter of CeO₂ nanoparticles was estimated by dynamic light scattering (DLS), using a Photocor Complex analyser. The microstructure of the samples was studied by transmission electron microscopy (TEM) with a Leo 912 AB Omega microscope at an accelerating voltage of 100 kV.

Preparation of linoleic acid and phosphatidylcholine hydroperoxides

To prepare a phosphate buffer solution (PBS, 100 mM pH 7.4), KH₂PO₄ (#P0662, Sigma-Aldrich) was used. A working solution of linoleic acid ($d = 0.9$ g cm⁻³, #L1376, Sigma-Aldrich) was

prepared by diluting the stock solution with dimethyl sulfoxide ($\geq 99.95\%$, #472301, Sigma-Aldrich). Oxidised linoleic acid (LOOH) was obtained by air barbotage.⁴³ The model phospholipid preparation was an ultrafine emulsion (nanoparticles up to 30 nm in size) containing 80–95% phosphatidylcholine and 20–5% maltose (Phospholipovit®, Institute of Biomedical Chemistry) dissolved in PBS (100 mM pH 7.4). Phosphatidylcholine hydroperoxide (PCOOH) was obtained by oxidation catalysed by lipoxygenase-1 (#P08170, Cayman).⁴⁴

The concentration of hydroperoxide groups in linoleic acid samples was determined by infrared (IR) spectroscopy.⁴⁵ The height of the absorption peak at the characteristic wavelength served as the analytical signal. The concentration of linoleic acid hydroperoxide (987 cm⁻¹, –O–O– group) in the linoleic acid samples was calculated from the calibration dependence for *tert*-butyl hydroperoxide (880 cm⁻¹, –O–O– group *t*-BuOOH).⁴⁵ The content of hydroperoxide in the samples of oxidised phosphatidylcholine was determined by HPLC with chemiluminescence detection.⁴⁶

Analysis of prooxidant activity of CeO₂ nanoparticles by the chemiluminescent method

Coumarin 334 (#393002, Sigma-Aldrich) was used as a chemiluminescent probe (CL probe) sensitive to lipid and phospholipid radicals. To obtain a working solution with a concentration of 5 mM, a weighed portion of coumarin 334 was dissolved in dimethyl sulfoxide ($\geq 99.95\%$, #472301, Sigma-Aldrich).

100 μ M hemoglobin solution (deoxyhemoglobin, Hb) stabilised with lithium heparin (17 IU mL⁻¹) was prepared by dissolving a weighed portion of lyophilised human hemoprotein powder (#H7379, Sigma-Aldrich) in distilled water in a vacuum blood collection tube (Vacutainer Plus). The exact concentration of Hb in the resulting solution was determined by optical spectroscopy, ϵ_{406} (Hb) = 270 548 cm⁻¹/M.

For comparative evaluation of the enzyme-like activity of citrate-stabilised CeO₂ sol, Ferinject® (Vifor Pharma, Fe(III) carboxymaltose), an iron-containing antianemic drug, was used. Working solutions were obtained by diluting the initial preparation (50 mg mL⁻¹) with distilled water.

Lipo- and phospholipoperoxidase-like activity of the samples was determined by recording the kinetics of free radical oxidation, according to a previously developed technique.⁴⁷ All measurements were carried out at room temperature.

Spontaneous chemiluminescence (CL) was recorded for 30–60 s in a system containing PBS (100 mM, pH 7.4), coumarin 334 (50 μ M) and model lipid or phospholipid substrates (hydroperoxides LOOH or PCOOH).⁴⁸ Then, using a chromatography syringe (Hamilton), an aliquot (50 μ L) of citrate-stabilised CeO₂ sol or solutions of deoxyhemoglobin or Fe(III) carboxymaltose was injected without interrupting the CL recording. This procedure allowed for the rapid recording of the kinetics of peroxidation processes (Fig. 1). Registration of the kinetics was performed for 5–7 min. As an analytical signal, the light sum was used, which is the area under the CL curve over a certain



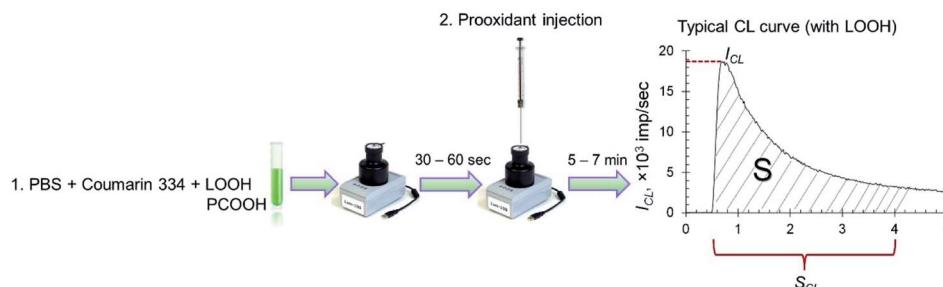


Fig. 1 Protocol for the determination of prooxidant activity towards lipid (LOOH) and phospholipid (PCOOH) hydroperoxides by the chemiluminescent method.

time interval (4 min) and is directly proportional to the number of formed radicals.

Results and discussion

Physicochemical characteristics of CeO_2 nanoparticles

Thermohydrolysis of an aqueous solution of ammonium cerium(IV) nitrate resulted in an electrostatically stabilised sol of nanocrystalline cerium dioxide. The content of CeO_2 in the sol was 23 g L^{-1} (0.13 M). According to XRD data (Fig. 2a) for the dried sol, it contained single-phase cerium dioxide (PDF2 34-0394). The particle size estimated by the Scherrer equation was 3 nm. The mean hydrodynamic diameter of CeO_2 nanoparticles obtained by the DLS method (Fig. 2b) was found to be 10–11 nm. The data on the particle size and phase composition of the obtained material were confirmed by the results of the analysis of the CeO_2 sol by TEM and electron diffraction (Fig. 2c).

The electron diffraction data and XRD analysis also confirm high degree of crystallinity of CeO_2 nanoparticles. The highly

crystalline nature of cerium oxide is consistent with recently reported study of citrate-stabilised ceria nanoparticles.⁴⁹

Lipoperoxidase-like activity of CeO_2 nanoparticles

The interaction of metal oxide nanoparticles with biomembranes is of key importance when considering their biological activity.^{50–52} Surprisingly, information on the redox activity of CeO_2 nanoparticles towards lipids and phospholipids is very scarce.^{25,53–55} This mainly consists of studies devoted to the interaction of CeO_2 nanoparticles with model membranes, *e.g.* lipid films or liposomes. At the same time, the rapid expansion of potential biomedical applications of CeO_2 nanoparticles makes it necessary to reveal their role in reactive oxygen species' homeostasis, including their interaction with oxidised lipoproteins of blood.

The prooxidant activity of citrate-stabilised CeO_2 sol was studied using the chemiluminescent method in a system containing linoleic acid hydroperoxide (LOOH) and coumarin 334, a highly selective lipid radical CL probe.^{56–58} The use of the

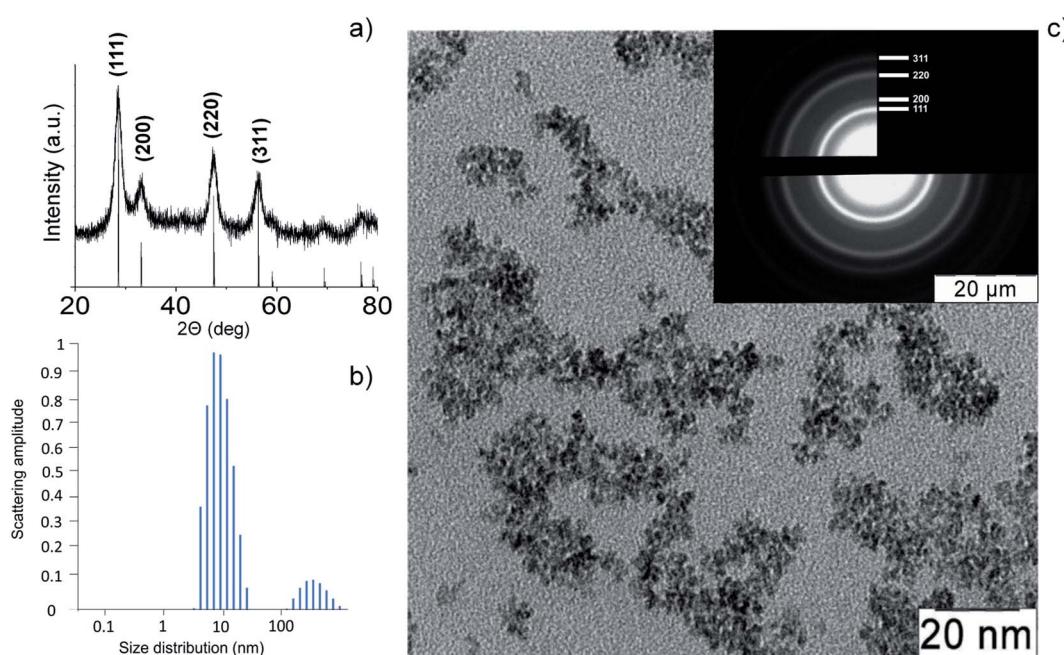


Fig. 2 Cerium oxide nanoparticles: (a) XRD pattern, (b) dynamic light scattering pattern, (c) TEM image and electron diffraction data (inset).

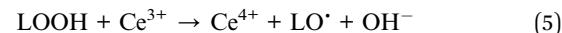
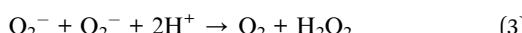
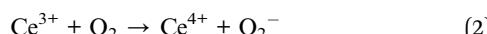
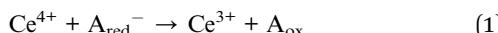


probe allows for the increase in the CL quantum yield and for the detection of free radical formation. According to a previously published protocol,⁴⁵ the concentration of hydroperoxide groups in LOOH samples estimated by IR spectroscopy was $100 \pm 12 \mu\text{mol L}^{-1}$.

The addition of various concentrations of a citrate-stabilised colloidal solution of CeO_2 nanoparticles to the mixed solution of LOOH and coumarin 334 resulted in a dose-dependent enhancement of chemiluminescence (Fig. 3a). The area under the curve (S_{CL}) was chosen as a measure of prooxidant capacity proportional to the number of formed reactive oxygen species per unit concentration of the prooxidant. The dependence of the analytical signal on the concentration of CeO_2 nanoparticles (Fig. 3b) was described by the equation $S_{\text{CL}} = (1.50 \pm 0.29) \times c$ (CeO_2 , mmol L^{-1}) + (16 ± 2) , $r = 0.995$ ($P = 0.95$, $n = 3$), where S_{CL} is the area under the corresponding CL curve, $\times 10^3$ imp, c (CeO_2) is the concentration of CeO_2 sol, mM .

The lipoperoxidase-like activity of nanodisperse cerium dioxide is demonstrated by the dose-dependent effect of chemiluminescence enhancement. A sharp increase in the intensity of chemiluminescence, followed by an exponential decay, which is characteristic of LPO, is additional evidence of lipid peroxidation induced by CeO_2 nanoparticles.^{57,59}

The data on the prooxidant activity of nanodisperse CeO_2 towards linoleic acid hydroperoxide are consistent with previously reported results, demonstrating the possible induction of lipid peroxidation by CeO_2 nanoparticles at the cellular and organismal levels.^{36,38,40} In these studies, the induction of LPO by CeO_2 nanoparticles was assessed by the level of various biomarkers, such as malondialdehyde, isoprostane, etc. To describe the LPO mechanism induced by CeO_2 nanoparticles (20 nm, 3.5, 10.5 and 23.3 $\mu\text{g mL}^{-1}$) on human bronchoalveolar carcinoma cell culture (A549), the following scheme was proposed:³⁶



where A_{red}^- are reducing agents (thiol-containing compounds, ascorbates), A_{ox} are their oxidised forms. As can be seen, in addition to H_2O_2 and OH^\cdot species formed as a result of reactions (1)–(4), lipid hydroperoxides interacting with CeO_2 nanoparticles also act as the sources of another type of radical, alkoxyl radicals, according to reaction (5). All these highly reactive particles (H_2O_2 , OH^\cdot , LO^\cdot) cause oxidative stress and, accordingly, LPO.

The induction of LPO after the treatment of cell cultures with nanodisperse cerium dioxide was also observed in the models of human hepatoma (SMMC-7721, CeO_2 NPs 20–30 nm; 50 $\mu\text{g mL}^{-1}$)³⁸ and human melanoma (A375, CeO_2 NPs ~ 38 nm; 20, 40, 80, 120 $\mu\text{g mL}^{-1}$).⁴⁰ Increasing the concentration of CeO_2 nanoparticles led to increased levels of the main LPO marker, malondialdehyde. In addition, preliminary treatment of the cells with antioxidants such as *N*-acetylcysteine significantly reduced the formation of ROS and malondialdehyde and facilitated the restoration of the activity of antioxidant enzymes.³⁸

The above *in vitro* studies with cancer cells demonstrated the fact that, in an acidic environment, CeO_2 nanoparticles lose their antioxidant (cytoprotective) activity and begin to function as a prooxidant, causing the development of oxidative stress, including LPO, and, subsequently, apoptosis. In the current study, lipoperoxidase-like activity of CeO_2 nanoparticles at pH 7.4 was observed. Analysis of existing data indicated that the redox activity of CeO_2 nanoparticles represents a set of closely related pro- and antioxidant properties determined by several factors, including the pH of the reaction medium, the method of preparation and the size of synthesised nanoparticles, the nature of surface ligands, charge, etc.⁶⁰ Park *et al.*, when studying the cytotoxicity of CeO_2 nanoparticles, proposed that cell type also affects the antioxidant action of ceria nanoparticles due to the factors associated with cell physiology.⁶¹ The results of the studies of CeO_2 nanoparticles' interaction with malignant cells suggest oxidative stress and LPO to be the most probable mechanisms of their toxicity.⁶² Thus, the lipoperoxidase-like activity of CeO_2 nanoparticles can be considered to be one of the key factors leading to adverse effects on the cells. The intravenous administration of an aqueous

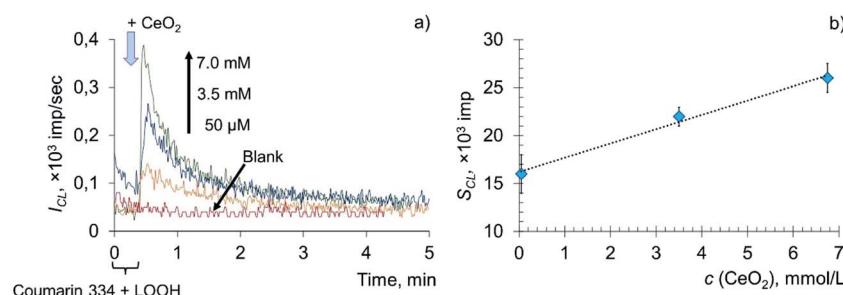


Fig. 3 Chemiluminograms of (a) citrate-stabilised CeO_2 sol in a phosphate buffer solution (100 mM, pH 7.4) + coumarin 334 (50 $\mu\text{mol L}^{-1}$) + LOOH (concentration of hydroperoxide groups is $100 \pm 12 \mu\text{mol L}^{-1}$); (b) the light sum dependence ($S_{\text{CL}} \times 10^3$ imp) on the concentration of CeO_2 sol.



dispersion of CeO_2 nanoparticles ($\sim 31 \pm 4$ nm) to rats has been reported to increase levels of an LPO product, 4-hydroxy-*trans*-2-nonenal, the highest concentrations of which have been found in the hippocampus.³⁹ Similarly, a high content of malondialdehyde in lung cells, indicating the toxic effect of CeO_2 nanoparticles (55 nm) and leading to membrane damage, has been found in rats exposed to CeO_2 -containing aerosol.³⁷ Increased LPO has been observed after intratracheal injection of a suspension of CeO_2 nanoparticles (~ 20 nm) to mice.⁴¹

The key role in the formation of radicals in blood plasma is played by the decomposition of lipid hydroperoxides by metal ions of variable valence (primarily Fe^{2+}) and hemoglobin. Thus, in this paper, deoxyhemoglobin (Hb), a natural ferrous compound, and Ferinject®, an antianemic drug containing $\text{Fe}(\text{III})$ in a stable form of a polynuclear iron hydroxide complex with a carbohydrate ligand, were chosen as model prooxidants.^{63–65} Experimental time dependences of chemiluminescence of the solutions containing deoxyhemoglobin and Ferinject® are shown in Fig. 4a and b, along with the corresponding concentration dependences (Fig. 4c and d).

The addition of Hb and $\text{Fe}(\text{III})$ carboxymaltose solutions to the system containing LOOH resulted in the rapid development of an intense chemiluminescence accompanying the lipoperoxidase-like reaction in a dose-dependent manner: $S_{\text{CL}} = (8.01 \pm 0.02) \times c$ (Hb, nM) + (249 ± 18) , $r = 0.996$, $n = 4$; $S_{\text{CL}} = (0.20 \pm 0.01) \times c$ ($\text{Fe}(\text{III})$, μM) + (5.15 ± 0.09) , $r = 0.992$, $n = 4$.

The data obtained indicate that citrate-stabilised CeO_2 sol (Fig. 3a), deoxyhemoglobin (Fig. 4a) and $\text{Fe}(\text{III})$ carboxymaltose (Fig. 4b) solutions induced linoleic acid hydroperoxide decomposition, while catalytic activity of these compounds differed significantly. Citrate-stabilised CeO_2 sol possessed significantly lower

prooxidant capacity (by more than six orders of magnitude) towards linoleic acid hydroperoxide than deoxyhemoglobin. In turn, the prooxidant capacity of Ferinject® preparation was two orders of magnitude higher than that of CeO_2 sol. This enables CeO_2 nanoparticles to be classified as a mild prooxidant agent. A possible reason for the relatively low lipoperoxidase-like activity of citrate-stabilised CeO_2 sol could be phosphate groups adsorbed on the surface of nanoparticles. This has been previously proven for other types of CeO_2 enzyme-like activities, namely SOD-like and catalase-like activities.^{66,67} In turn, the similarity of the redox properties of citrate-stabilised CeO_2 sol and Ferinject® to hydrogen peroxide (Fig. S1 in ESI†) can be partially explained by their common nature. The latter is also a colloidal solution of 20 nm nanoparticles which consists of a polynuclear iron(III)-oxyhydroxide core stabilised by carboxymaltose.⁶⁴ Comparison of the kinetics of lipid hydroperoxide decomposition in the presence of CeO_2 nanoparticles (Fig. 3) with previously obtained data on the CeO_2 -assisted decomposition kinetics of hydrogen peroxide (Fig. S2 in ESI†)⁶⁸ enables the suggestion of similar mechanisms of the prooxidant activity of CeO_2 nanoparticles towards different substrates.

Data on the prooxidant properties of CeO_2 nanoparticles are apparently in contrast to numerous results demonstrating their cytoprotective activity.^{19,69,70} The inhibitory effect of CeO_2 on LPO suggests the dual nature of its regulatory function. For example, the inhibition of LPO by CeO_2 nanoparticles has been shown in a model of the dominant retinitis pigmentosa of rats (P23H-1, 3–5 nm CeO_2 NPs; 344 ng per eye),²⁴ in a model of hypobaric hypoxia (Sprague Dawley rat line, 7–10 nm CeO_2 -NPs),⁷¹ in an *in vitro* model of diabetic ketoacidosis (HepG2,

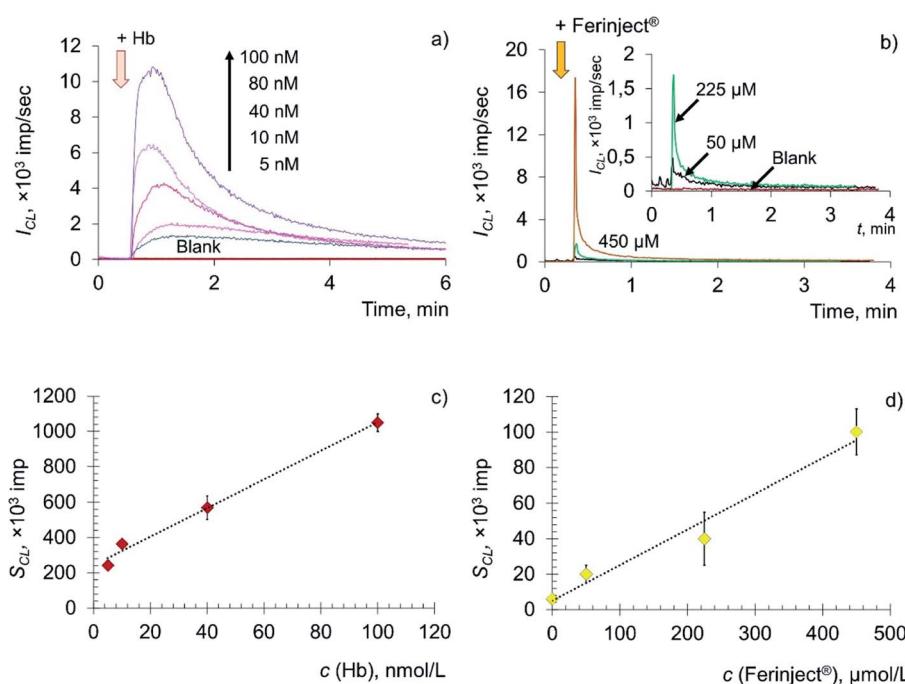


Fig. 4 Chemiluminograms of (a) an aqueous solution of deoxyhemoglobin (Hb) and (b) a colloidal solution of $\text{Fe}(\text{III})$ carboxymaltose (Ferinject®) in a phosphate buffer solution (100 mM, pH 7.4) + coumarin 334 (50 $\mu\text{mol L}^{-1}$) + LOOH (concentration of hydroperoxide groups was 100 ± 12 $\mu\text{mol L}^{-1}$); (c) and (d) light sum dependences ($S_{\text{CL}} \times 10^3$ imp) on the concentration of deoxyhemoglobin solutions and Ferinject®, respectively.



CeO_2 NPs)⁷² and in a study of nephrotoxicity caused by Cisplatin® (113 ± 14 nm CeO_2 NPs).⁷³

Thus, existing data make possible the suggestion that different concentrations of CeO_2 nanoparticles provide either an activating or inhibiting effect on LPO. Results of the current research demonstrate that citrate-stabilised CeO_2 sol, at 0.05–7.0 mM concentrations, in a model of LOOH oxidation, exhibits prooxidant properties only, being an LPO inducer. Among the factors that change the action of substances from being antioxidant to being prooxidant are the presence of other metal ions, the concentration of the substance and its redox potential.^{74,75} For example, Fe^{2+} ions can act as both pro- and anti-oxidants. The dual role of Fe^{2+} ions in chain peroxidation reactions is due to the fact that they react with both hydroperoxides and free radicals.^{76,77} Interacting with the former, Fe^{2+} ions promote branching of the lipid oxidation chains and thereby activate LPO, which in this case is a prooxidant. In turn, when reacting with free radicals, Fe^{2+} ions act as an antioxidant. At each site of the chain reaction, the lipoperoxyl radical leading the chain can react with either a new lipid molecule or an inhibitor, (in this case, Fe^{2+} ions). When the probability of the former process is higher, the chain reaction proceeds with self-acceleration; when the latter process prevails, the chain reaction proceeds with deceleration. Thus, the concentration of $\text{Fe}(\text{II})$ species plays a determining role in LPO induction. The similar exponential decay kinetics (and, most probably, similar mechanisms) of lipid peroxidation induced by CeO_2 nanoparticles, deoxyhemoglobin ($\text{Fe}(\text{II})$) and $\text{Fe}(\text{III})$ carboxymaltose (Ferinject®) suggest CeO_2 concentration to be one of the factors determining whether there is a prooxidant or antioxidant effect of CeO_2 nanoparticles on lipid peroxidation.

Phospholipoperoxidase-like activity of CeO_2 nanoparticles

Phospholipids play multiple roles in cells. Phospholipids not only modulate membrane permeability and act as their supporting scaffold, but also participate in signal transduction in response to external and internal stimuli.^{28,78–80} In addition, they are the source of arachidonic acid and other polyunsaturated fatty acids, from which, as a result of a number of metabolic reactions, lipid mediators are formed that regulate various biological functions.^{80,81} It should be noted that phospholipids are the major targets of reactive oxygen species attack under conditions of oxidative stress.⁸² In this regard, the biochemical activity of CeO_2 nanoparticles towards the key component of the cell membrane, phospholipids, was also investigated.

The prooxidant activity of citrate-stabilised CeO_2 sol was analysed by a chemiluminescence method in a biochemical model containing oxidised phosphatidylcholine (PCOOH) and coumarin 334. Amounts of hydroperoxide groups in the phospholipid substrate were determined according to an established protocol⁴⁶ and were found to be 98 ± 10 $\mu\text{mol L}^{-1}$. An aqueous solution of deoxyhemoglobin ($\text{Fe}(\text{II})$) was used as a reference sample.

The addition of citrate-stabilised CeO_2 sol to the solution of phosphatidylcholine hydroperoxide and coumarin 334 led to a dose-dependent increase in chemiluminescence, which

reached a stationary level and then gradually decreased (Fig. 5a). The dependence of the CL signal on the concentration of CeO_2 sol (Fig. 5b) was described by the equation: $S_{\text{CL}} = (22 \pm 3) \times c (\text{CeO}_2, \mu\text{M}) + (35 \pm 5)$, $r = 0.997$, $n = 3$. In a similar way, chemiluminograms were recorded for deoxyhemoglobin (Fig. 5c). A dose-dependent increase in luminescence intensity was observed (Fig. 5d): $S_{\text{CL}} = (552 \pm 80) \times c (\text{Hb}, \mu\text{M}) + (752 \pm 119)$, $r = 0.976$, $n = 3$.

The dose-dependent enhancement of chemiluminescence caused by the addition of CeO_2 nanoparticles and Hb solution to the phospholipid substrate suggests their phospholipoperoxidase-like activity. Presumably, the mechanism of interaction of Hb with PCOOH is similar to the mechanism of oxidation of hydroperoxides (LOOH) induced by metal ions of variable valence: $\text{PCOOH} \rightarrow \text{PCO}^\cdot + \text{breakdown products}$.⁸³ However, the kinetics of catalytic decomposition of PCOOH induced by CeO_2 nanoparticles and Hb developed much more slowly in comparison with the oxidation of lipid hydroperoxide (LOOH).

To assess the quantitative differences in activity of CeO_2 nanoparticles and Hb, the prooxidant capacity of deoxyhemoglobin was taken as 1. It was found that citrate-stabilised CeO_2 sol possessed approximately 20 times less prooxidant capacity in the reaction of phosphatidylcholine hydroperoxide peroxidation than deoxyhemoglobin.

In the systems containing lipid and phospholipid hydroperoxides, CeO_2 nanoparticles demonstrated significantly lower prooxidant activity compared to hemoprotein. The dependence of the CL signal on the concentration of citrate-stabilised CeO_2 sol and Hb in the system containing LOOH was linear (Fig. 6a), whereas in the system containing phospholipid hydroperoxide the dependence was of a complex nature (Fig. 6b).

There are several factors that determine the switching of the lipid peroxidation process from self-accelerating to decelerating when the substrate is phosphatidylcholine hydroperoxide. Phosphatidylcholines (1,2-diacyl-*sn*-glycero-3-phosphocholines, lecithins) have a zwitter-ionic structure in a wide pH range.⁸⁴ Important factors affecting the oxidation of phospholipids are the size and composition of liposomes, the nature of the prooxidant and the acyl chain that determines the selectivity of substrate modification.⁸² Recently, kinetic patterns of egg lecithin oxidation in its liposomal aqueous solutions were reported.⁸⁴ It was found that the oxidation rate was proportional to the radical initiation rate and depended nonlinearly on phospholipid concentration.⁸⁴ This is explained by the fact that, in a microheterogeneous environment, along with individual radicals and molecules, microaggregates (liposomes) also exist, whose reactivity differs from that of molecular species.⁸⁴

In the current study, differences in the reaction kinetics for systems containing lipid and phospholipid hydroperoxides were probably due to their nature. In oil-in-water emulsions, the oxidation rate of lipid substrates is strongly influenced by the properties of the interface, determining the rate of the interaction of water-soluble prooxidants with hydroperoxides localised within, and on, the boundary of the emulsion drop.^{84–86} Visually, the linoleic acid hydroperoxide sample is an emulsion in a phosphate buffer solution, whereas PCOOH is



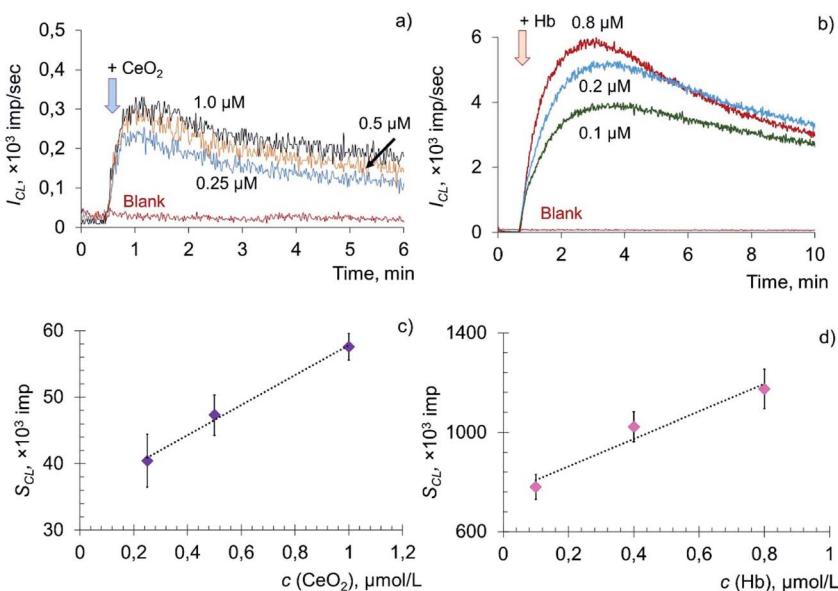


Fig. 5 Chemiluminograms of (a) citrate-stabilised CeO₂ sol and (b) deoxyhemoglobin (Hb) solution in a phosphate buffer solution (100 mM, pH 7.4) + coumarin 334 (50 $\mu\text{mol L}^{-1}$) + PCOOH (concentration of hydroperoxide groups is $98 \pm 10 \mu\text{mol L}^{-1}$); (c) and (d) light sum dependences ($S_{CL} \times 10^3$ imp) on the concentration of CeO₂ sol and deoxyhemoglobin solution, respectively.

a homogeneous solution formed as a result of an oxidative reaction catalysed by lipoxygenase-1 from an ultradispersed emulsion of phosphatidylcholine (with nanoparticles as small as 30 nm).

A comparison of chemiluminescence curves obtained for CeO₂ nanoparticles and Fe(III) carboxymaltose (Ferinject®), in the presence of PCOOH, is provided in Fig. 7.

The prooxidant capacity of citrate-stabilised CeO₂ sol was four times higher than that of a colloidal solution of Fe(III) carboxymaltose. It should be noted that the ammonium citrate did not contribute to the enhancement of chemiluminescence when CeO₂ nanoparticles were added (Fig. S3 in ESI†).

To fully understand the biological activities taking place, and to expand the field of biomedical applications for CeO₂ nanzymes, the mechanisms of nanoparticle–membrane interactions need to be analysed. There are only a few works devoted to the redox activity of nanodisperse CeO₂ towards phospholipids. Khulbe *et al.* focused their attention on the phospholipase-like

(phospholipase C) mimetic activity of polymer-coated CeO₂ nanoparticles.¹⁶ CeO₂ nanoparticles demonstrated bactericidal activity, causing membrane disruption in a wide range of pathogens, including respiratory pathogens (*Klebsiella pneumoniae*) and biofilm-forming bacteria.¹⁶ Important data on the molecular mechanisms of CeO₂ nanoparticles' interaction with the cell membrane model were obtained by Liu *et al.*,⁵³ who showed that the interaction of CeO₂ nanoparticles with phosphocholine liposomes composed of two different types of phospholipid, DOPC and DPPC, proceeds mainly *via* the phosphate groups in the phospholipids.⁵³

The present study demonstrates that citrate-stabilised CeO₂ sol exhibits prooxidant activity towards organic hydroperoxides that were different in nature. The phospholipoperoxidase-like activity of CeO₂ nanoparticles was notably higher than their lipoperoxidase-like activity (by approximately 25 times, see also ESI†). This confirms the special tropism of CeO₂ nanoparticles to phospholipids. Recently, CeO₂ enzyme-like activity was reported to

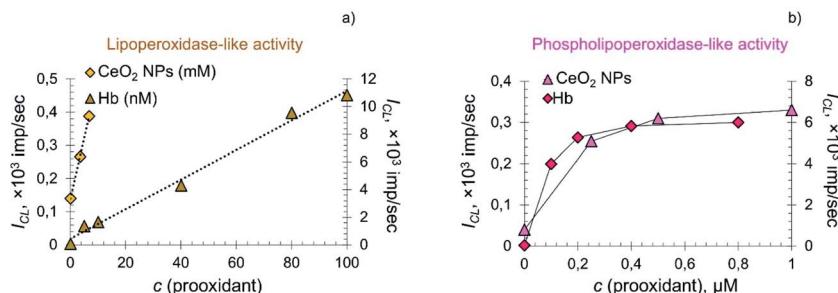


Fig. 6 Dependences of chemiluminescence intensity ($I_{CL} \times 10^3$ imp/s) on the concentration of prooxidant (citrate-stabilised CeO₂ sol or deoxyhemoglobin) added to the solution containing phosphate buffer solution (100 mM, pH 7.4) + coumarin 334 (50 $\mu\text{mol L}^{-1}$) + (a) LOOH ($100 \pm 12 \mu\text{mol L}^{-1}$) or (b) PCOOH (concentration of hydroperoxide groups was $98 \pm 10 \mu\text{mol L}^{-1}$).

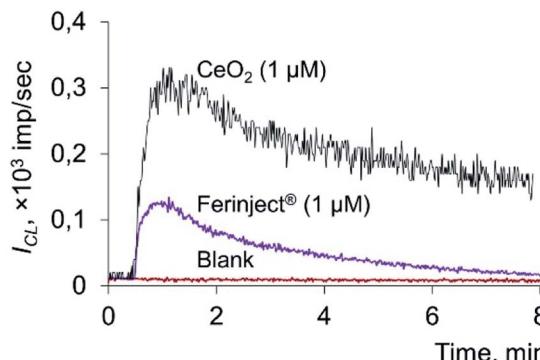


Fig. 7 Chemiluminograms of citrate-stabilised CeO_2 sol and a colloidal solution of carboxymaltose $\text{Fe}(\text{III})$ (Ferinject®) in a phosphate buffer solution (100 mM, pH 7.4) + coumarin 334 ($50 \mu\text{mol L}^{-1}$) + PCOOH (concentration of hydroperoxide groups was $98 \pm 10 \mu\text{mol L}^{-1}$).

depend strongly on particle size, most probably due to the differences in surface atoms concentration and cerium valence state.^{39,87,88} Generally speaking, for smaller CeO_2 nanoparticles, the catalytic properties were found to be more pronounced.^{12,89,90} In the present study, new types of enzyme-like activities of CeO_2 NPs were discovered, which are also expected to be dependent on ceria particle size in the same manner. However, the influence of the nanoparticles' size on their catalytic properties may be quite different. For example, Liu *et al.* showed that, especially for the peroxidase mimicking activity, smaller particles do not always exhibit higher catalytic activity.⁴⁹

Temperature is an important parameter affecting the activity of enzymes, thus it was necessary to investigate the enzyme-like activities of nanocrystalline ceria at biologically relevant temperatures, too. At 37°C , the lipo- and phospholipoperoxidase-like activities of CeO_2 nanoparticles were higher than at 25°C , by approximately $40 \pm 2\%$ (see Fig. S4 and S5 in ESI†). Interestingly, when the temperature was increased from 25 to 37°C , lipo- and phospholipoperoxidase-like activity of deoxyhemoglobin increased to a markedly higher extent. These observations are almost in line with the milder prooxidant properties of nanoceria compared to deoxyhemoglobin and the higher sensitivity of protein catalysts to the influence of temperature than inorganic catalysts.

Analysis of the kinetics of lipid peroxidation induced by CeO_2 nanoparticles, deoxyhemoglobin ($\text{Fe}(\text{II})$) and $\text{Fe}(\text{III})$ carboxymaltose (Ferinject®) enabled the assumption of similar mechanisms in relation to their prooxidant action. That is, nanoceria-induced lipid or phospholipid peroxidation could be similar to conventional iron-assisted peroxidation (Fig. 8).^{57,59,77,91} CeO_2 NPs catalyse oxidation of both linoleic acid and phosphatidylcholine peroxides, and free radicals are consequently formed. The key difference between these catalytic reactions are different reaction rates, phospholipid peroxidation proceeding slower than lipid peroxidation.

New types of enzyme-like activities of ceria additionally expand the scope of its practical applications. In particular, artificial enzymes possessing specific activity can be used for fine tuning enzyme-catalysed processes in living cells.



Fig. 8 Schematic diagram showing the nanzyme activities of CeO_2 nanoparticles towards oxidised lipids and phospholipids (LOOH = linoleic acid hydroperoxide, PCOOH = phosphatidylcholine hydroperoxide).

Non-enzymatic lipid oxidation is usually considered deleterious, while enzymatic peroxidation is considered beneficial for cell survival and generates relatively few lipid regulators. However, both processes often proceed simultaneously and may influence each other. Recent studies have demonstrated that lipid peroxidation products play an important role in signalling^{92,93} through reactive carbonyls such as malonic dialdehyde and 4-hydroxynonenal, which are able to react with proteins and modify them.^{94–96} Lipid aldehydes activate proinflammatory NF- κB signalling pathways.⁹⁷ Non-enzymatic inducers of lipid peroxidation, such as iron or copper complexes, react with lipid hydroperoxides, phospholipid peroxides and hydrogen peroxide. Since CeO_2 NPs exhibit mostly phospholipoperoxidase-like activity, they can act as a mild selective agent for phospholipid peroxidation. Such an artificial triggering of LPO-dependent signalling pathways would allow for a deeper insight into pathological processes in the cells. On the other hand, a relatively low lipoperoxidase-like activity is beneficial for the possible use of nanoceria as a theranostic agent.^{98,99}

Conclusions

In the present study, CeO_2 nanoparticles were shown to possess two novel types of enzyme-like activity, namely lipoperoxidase and phospholipoperoxidase activity. In the presence of ceria NPs, organic peroxide substrates (linoleic acid hydroperoxide and phosphatidylcholine hydroperoxide) decomposed catalytically to form free radicals. The prooxidant capacity of CeO_2 was quantitatively assessed using a chemiluminescent method with a coumarin 334 luminescent probe. Lipoperoxidase-like activity of nanoceria appeared to be lower than phospholipoperoxidase-like activity, probably due to the higher tropism of ceria towards phosphate groups. Both phospholipoperoxidase-like and lipoperoxidase-like activities of nanoceria were significantly lower than that of deoxyhemoglobin. Thus, CeO_2 nanoparticles can act as a mild selective agent for phospholipid peroxidation.

Mild lipoperoxidase- and phospholipoperoxidase-like activities are advantageous for the possible biomedical applications of CeO_2 nanoparticles as a lipid and phospholipid radical regulator in living systems.

Author contributions

Conceptualisation, E. V. P. and V. K. I.; methodology, M. M. S., E. V. P. and V. K. I.; data curation, E. V. P.; investigation, M. M. S. and A. L. P.; validation, M. M. S.; supervision, V. K. I.; funding acquisition, M. M. S.; project administration, V. K. I.; resources, A. L. K.; writing—original draft preparation, M. M. S.; writing—



review and editing, E. V. P. and V. K. I. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors have no conflicts of interest to disclose.

Acknowledgements

This work was supported by the Russian Science Foundation (project 21-73-00251).

References

- D. Jiang, D. Ni, Z. T. Rosenkrans, P. Huang, X. Yan and W. Cai, *Chem. Soc. Rev.*, 2019, **48**, 3683–3704.
- H. Wang, K. Wan and X. Shi, *Adv. Mater.*, 2019, **31**, 1805368.
- R. Zhang, K. Fan and X. Yan, *Sci. China: Life Sci.*, 2020, **63**, 1183–1200.
- T. Kang, Y. G. Kim, D. Kim and T. Hyeon, *Coord. Chem. Rev.*, 2020, **403**, 213092.
- X. Liu, Y. Gao, R. Chandrawati and L. Hosta-Rigau, *Nanoscale*, 2019, **11**, 21046–21060.
- Y. Huang, J. Ren and X. Qu, *Chem. Rev.*, 2019, **119**, 4357–4412.
- X. Wang, W. Guo, Y. Hu, J. Wu and H. Wei, *Nanozymes: next wave of artificial enzymes*, Springer International Publishing, 2016.
- C. Korsvik, S. Patil, S. Seal and W. T. Self, *Chem. Commun.*, 2007, 1056–1058.
- E. G. Heckert, A. S. Karakoti, S. Seal and W. T. Self, *Biomaterials*, 2008, **29**, 2705–2709.
- M. M. Sozarukova, M. A. Shestakova, M. A. Teplonogova, D. Y. Izmailov, E. V. Proskurnina and V. K. Ivanov, *Russ. J. Inorg. Chem.*, 2020, **65**, 597–605.
- T. Pirmohamed, J. M. Dowding, S. Singh, B. Wasserman, E. Heckert, A. S. Karakoti, J. E. King, S. Seal and W. T. Self, *Chem. Commun.*, 2010, **46**, 2736–2738.
- A. Asati, S. Santra, C. Kaittanis, S. Nath and J. M. Perez, *Angew. Chem., Int. Ed.*, 2009, **48**, 2308–2312.
- B. Liu, Z. Huang and J. Liu, *Nanoscale*, 2016, **8**, 13562–13567.
- T. Yao, Z. Tian, Y. Zhang and Y. Qu, *ACS Appl. Mater. Interfaces*, 2019, **11**, 195–201.
- Z. Tian, T. Yao, C. Qu, S. Zhang, X. Li and Y. Qu, *Nano Lett.*, 2019, **19**, 8270–8277.
- K. Khulbe, K. Karmakar, S. Ghosh, K. Chandra, D. Chakravortty and G. Muges, *ACS Appl. Bio Mater.*, 2020, 1–36.
- F. Xu, Q. Lu, P. J. Huang and J. Liu, *Chem. Commun.*, 2019, **55**, 13215–13218.
- A. B. Shcherbakov, V. V. Reukov, A. V. Yakimansky, E. L. Krasnopalova, O. S. Ivanova, A. L. Popov and V. K. Ivanov, *Polymers*, 2021, **13**, 924.
- A. B. Shcherbakov, N. M. Zholobak and V. K. Ivanov, in *Cerium Oxide (CeO₂): Synthesis, Properties and Applications*, ed. S. Scirè and L. Palmisano, Elsevier, Amsterdam, 2020, pp. 279–358.
- S. Rajeshkumar and P. Naik, *Biotechnol. Rep.*, 2018, **17**, 1–5.
- A. Dhall and W. Self, *Antioxidants*, 2018, **7**, 97.
- H. J. Kwon, M.-Y. Cha, D. Kim, D. K. Kim, M. Soh, K. Shin, T. Hyeon and I. Mook-Jung, *ACS Nano*, 2016, **10**, 2860–2870.
- M. Hijaz, S. Das, I. Mert, A. Gupta, Z. Al-Wahab, C. Tebbe, S. Dar, J. Chhina, S. Giri and A. Munkarah, *BMC Cancer*, 2016, **16**, 1–14.
- L. L. Wong, Q. N. Pye, L. Chen, S. Seal and J. F. McGinnis, *PLoS One*, 2015, **10**, e0121977.
- A. Kumar, S. Das, P. Munusamy, W. Self, D. R. Baer, D. C. Sayle and S. Seal, *Environ. Sci.: Nano*, 2014, **1**, 516–532.
- T. Xia, M. Kovochich, M. Lioung, L. Madler, B. Gilbert, H. Shi, J. I. Yeh, J. I. Zink and A. E. Nel, *ACS Nano*, 2008, **2**, 2121–2134.
- Z. Ji, X. Wang, H. Zhang, S. Lin, H. Meng, B. Sun, S. George, T. Xia, A. E. Nel and J. I. Zink, *ACS Nano*, 2012, **6**, 5366–5380.
- R. Pamplona, *Biochim. Biophys. Acta, Bioenerg.*, 2008, **1777**, 1249–1262.
- M. Ikeda, A. Kihara and Y. Igarashi, *Biol. Pharm. Bull.*, 2006, **29**, 1542–1546.
- G. Lenoir, P. Williamson and J. C. Holthuis, *Curr. Opin. Chem. Biol.*, 2007, **11**, 654–661.
- M. Portero-Otín, M. J. Bellumunt, M. C. Ruiz, G. Barja and R. Pamplona, *Lipids*, 2001, **36**, 491–498.
- G. Van Meer, D. R. Voelker and G. W. Feigenson, *Nat. Rev. Mol. Cell Biol.*, 2008, **9**, 112–124.
- E. Niki, *Biofactors*, 2008, **34**, 171–180.
- H. Yin, L. Xu and N. A. Porter, *Chem. Rev.*, 2011, **111**, 5944–5972.
- L. J. Su, J. H. Zhang, H. Gomez, R. Murugan, X. Hong, D. Xu, F. Jiang and Z. Y. Peng, *Oxid. Med. Cell. Longevity*, 2019, **2019**, 5080843.
- W. Lin, Y. W. Huang, X. D. Zhou and Y. Ma, *Int. J. Toxicol.*, 2006, **25**, 451–457.
- A. Srinivas, P. J. Rao, G. Selvam, P. B. Murthy and P. N. Reddy, *Toxicol. Lett.*, 2011, **205**, 105–115.
- G. Cheng, W. Guo, L. Han, E. Chen, L. Kong, L. Wang, W. Ai, N. Song, H. Li and H. Chen, *Toxicol. In Vitro*, 2013, **27**, 1082–1088.
- R. A. Yokel, M. T. Tseng, M. Dan, J. M. Unrine, U. M. Graham, P. Wu and E. A. Grulke, *Nanomedicine*, 2013, **9**, 398–407.
- D. Ali, S. Alarifi, S. Alkahtani, A. A. AlKahtane and A. Almalik, *Cell Biochem. Biophys.*, 2015, **71**, 1643–1651.
- A. Nemmar, P. Yuvaraju, S. Beegam, M. A. Fahim and B. H. Ali, *Oxid. Med. Cell. Longevity*, 2017, **2017**, 9639035.
- A. B. Shcherbakov, M. A. Teplonogova, O. S. Ivanova, T. O. Shekunova, I. V. Ivonin, A. E. Baranchikov and V. K. Ivanov, *Mater. Res. Express*, 2017, 055008.
- S. Adachi, T. Ishiguro and R. Matsuno, *J. Am. Oil Chem. Soc.*, 1995, **72**, 547.
- J. Ito, K. Nakagawa, S. Kato, T. Hirokawa, S. Kuwahara, T. Nagai and T. Miyazawa, *J. Chromatogr. A*, 2015, **1386**, 53–61.
- A. A. Dzhatdoeva, A. M. Polimova, E. V. Proskurnina, M. A. Proskurnin and Y. A. Vladimirov, *J. Anal. Chem.*, 2016, **71**, 542–548.
- T. Miyazawa, T. Suzuki, K. Fujimoto and K. Yasuda, *J. Lipid Res.*, 1992, **33**, 1051–1059.



- 47 E. V. Proskurnina, A. A. Dzhatdoeva, E. S. Lobichenko, R. I. Shalina and Y. A. Vladimirov, *J. Anal. Chem.*, 2017, **72**, 751–755.
- 48 P. O. Volkova, A. V. Alekseev, A. A. Dzhatdoeva, E. V. Proskurnina and Y. A. Vladimirov, *Moscow Univ. Chem. Bull.*, 2016, **71**, 87–96.
- 49 X. Liu, J. Wu, Q. Liu, A. Lin, S. Li, Y. Zhang, Q. Wang, T. Li, X. An and Z. Zhou, *J. Mater. Chem. B*, 2021, **9**, 7238–7245.
- 50 A. E. Nel, L. Mädler, D. Velegol, T. Xia, E. M. Hoek, P. Somasundaran, F. Klaessig, V. Castranova and M. Thompson, *Nat. Mater.*, 2009, **8**, 543–557.
- 51 A. Verma and F. Stellacci, *Small*, 2010, **6**, 12–21.
- 52 J. Liu, *Langmuir*, 2016, **32**, 4393–4404.
- 53 Y. Liu and J. Liu, *Langmuir*, 2016, **32**, 13276–13283.
- 54 B. Foster, M. Larios and V. Smith, *FASEB J.*, 2016, **30**, lb74.
- 55 M. H. Wong, R. P. Misra, J. P. Giraldo, S.-Y. Kwak, Y. Son, M. P. Landry, J. W. Swan, D. Blankschtein and M. S. Strano, *Nano Lett.*, 2016, **16**, 1161–1172.
- 56 Y. A. Vladimirov, V. S. Sharov, E. S. Driomina, A. V. Reznitchenko and S. B. Gashev, *Free Radical Biol. Med.*, 1995, **18**, 739–745.
- 57 Y. A. Vladimirov, V. S. Sharov, E. S. Driomina, A. V. Reznitchenko and S. B. Gashev, *Free Radical Biol. Med.*, 1995, **18**, 739–745.
- 58 N. Baker, G. Greenway, R. Wheatley and C. Wiles, *Analyst*, 2007, **132**, 104–106.
- 59 Y. A. Vladimirov, E. V. Proskurina and D. Yu. Izmailov, *Biofizika*, 2011, **56**, 1081–1090.
- 60 A. Asati, S. Santra, C. Kaittanis and J. M. Perez, *ACS Nano*, 2010, **4**, 5321–5331.
- 61 E. J. Park, J. Choi, Y. K. Park and K. Park, *Toxicology*, 2008, **245**, 90–100.
- 62 A. Nel, T. Xia, L. Madler and N. Li, *Science*, 2006, **311**, 622–627.
- 63 R. A. Moore, H. Gaskell, P. Rose and J. Allan, *BMC Blood Disord.*, 2011, **11**, 4.
- 64 L. J. Scott, *Drugs*, 2018, **78**, 479–493.
- 65 B. Dalzon, A. Torres, S. Reymond, B. Gallet, F. Saint-Antonin, V. Collin-Faure, C. Moriscot, D. Fenel, G. Schoehn, C. Aude-Garcia and T. Rabilloud, *Nanomaterials*, 2020, **10**, 266.
- 66 R. N. McCormack, P. Mendez, S. Barkam, C. J. Neal, S. Das and S. Seal, *J. Phys. Chem. C*, 2014, **118**, 18992–19006.
- 67 Y. Zhao, H. Li, A. Lopez, H. Su and J. Liu, *ChemBioChem*, 2020, **21**, 2178–2186.
- 68 M. M. Sozarukova, E. V. Proskurnina, A. E. Baranchikov and V. K. Ivanov, *Nanosyst.: Phys., Chem., Math.*, 2020, **11**, 324–332.
- 69 B. C. Nelson, M. E. Johnson, M. L. Walker, K. R. Riley and C. M. Sims, *Antioxidants*, 2016, **5**, 15.
- 70 R. Zhang, K. Fan and X. Yan, in *Nanozymology: Connecting Biology and Nanotechnology*, ed. X. Yan, Springer, 2020, ch. 9, pp. 279–329.
- 71 A. Arya, N. K. Sethy, S. K. Singh, M. Das and K. Bhargava, *Int. J. Nanomed.*, 2013, **8**, 4507–4520.
- 72 M. Shokrzadeh, H. Abdi, A. Asadollah-Pour and F. Shaki, *Cell J.*, 2016, **18**, 97–102.
- 73 M. A. Saifi, S. Sangomla, A. Khurana and C. Godugu, *Biol. Trace Elem. Res.*, 2019, **189**, 145–156.
- 74 B. Poljsak and P. Raspot, *J. Appl. Toxicol.*, 2008, **28**, 183–188.
- 75 R. Sotler, B. Poljsak, R. Dahmane, T. Jukic, D. Pavan Jukic, C. Rotim, P. Trebse and A. Starc, *Acta Clin. Croat.*, 2019, **58**, 726–736.
- 76 T. B. Suslova, V. I. Olenev, M. V. Korchagina and I. A. Vladimirov, *Biofizika*, 1970, **15**, 622–628.
- 77 Y. A. Vladimirov and A. A. Archakov, *Lipid Peroxidation in Biological Membranes* [in Russian], Nauka, Moscow, 1972.
- 78 W. Dowhan, *J. Biol. Chem.*, 2017, **292**, 10755–10766.
- 79 E. M. Mejia and G. M. Hatch, *J. Bioenerg. Biomembr.*, 2016, **48**, 99–112.
- 80 C. Solís-Calero, J. Ortega-Castro, J. Frau and F. Muñoz, *Oxid. Med. Cell. Longevity*, 2015, **2015**, 319505.
- 81 H. Tallima and R. El Ridi, *J. Adv. Res.*, 2018, **11**, 33–41.
- 82 A. Reis and C. M. Spickett, *Biochim. Biophys. Acta*, 2012, **1818**, 2374–2387.
- 83 Y. Bao and G. Williamson, *Redox Rep.*, 1997, **3**, 325–330.
- 84 E. A. Mengele, I. G. Plashchina and O. T. Kasaikina, *Colloid J.*, 2011, **73**, 815–821.
- 85 W. Chaiyasit, M. Silvestre, D. J. McClements and E. A. Decker, *J. Agric. Food Chem.*, 2000, **48**, 3077–3080.
- 86 U. Klinkesorn, P. Sophanodora, P. Chinachoti, D. J. McClements and E. A. Decker, *J. Agric. Food Chem.*, 2005, **53**, 4561–4566.
- 87 J. Gagnon and K. M. Fromm, *Eur. J. Inorg. Chem.*, 2015, **2015**, 4510–4517.
- 88 G. Song, N. Cheng, J. Zhang, H. Huang, Y. Yuan, X. He, Y. Luo and K. Huang, *Catalysts*, 2021, **11**, 1123.
- 89 S. S. Lee, W. Song, M. Cho, H. L. Puppala, P. Nguyen, H. Zhu, L. Segatori and V. L. Colvin, *ACS Nano*, 2013, **7**, 9693–9703.
- 90 V. Baldim, F. Bedioui, N. Mignet, I. Margaill and J.-F. Berret, *Nanoscale*, 2018, **10**, 6971–6980.
- 91 Y. A. Vladimirov, V. I. Olenev, T. B. Suslova and Z. P. Cheremisina, *Adv. Lipid Res.*, 1980, **17**, 173–249.
- 92 S. Albadri, F. Naso, M. Thauvin, C. Gauron, C. Parolin, K. Duroure, J. Vougy, J. Fiori, C. Boga, S. Vriz, N. Calonghi and F. Del Bene, *Dev. Cell*, 2019, **50**, 73–89.
- 93 C. Kruger, S. J. Burke, J. J. Collier, T. T. Nguyen, J. M. Salbaum and K. Stadler, *Redox Biol.*, 2018, **16**, 248–254.
- 94 A. Ayala, M. F. Munoz and S. Arguelles, *Oxid. Med. Cell. Longevity*, 2014, **2014**, 360438.
- 95 T. D. Calamaras, C. Lee, F. Lan, Y. Ido, D. A. Siwik and W. S. Colucci, *Free Radical Biol. Med.*, 2015, **82**, 137–146.
- 96 Y. Yang, R. Sharma, A. Sharma, S. Awasthi and Y. C. Awasthi, *Acta Biochim. Pol.*, 2003, **50**, 319–336.
- 97 U. C. Yadav and K. V. Ramana, *Oxid. Med. Cell. Longevity*, 2013, **2013**, 690545.
- 98 P. Eriksson, A. A. Tal, A. Skallberg, C. Brommesson, Z. Hu, R. D. Boyd, W. Olovsson, N. Fairley, I. A. Abrikosov and X. Zhang, *Sci. Rep.*, 2018, **8**, 1–12.
- 99 J. Xie, S. Lee and X. Chen, *Adv. Drug Delivery Rev.*, 2010, **62**, 1064–1079.

