

Electron paramagnetic study on radical scavenging properties of ceria nanoparticles

Suresh Babu ^a, Anthony Velez ^{b,1}, Krzysztof Wozniak ^b, Jadwiga Szydłowska ^b,
Sudipta Seal ^{a,*}

^a Department of Mechanical, Materials and Aerospace Engineering, Advanced Materials Processing and Analysis Centre,
University of Central Florida, Orlando, FL 32816, USA

^b Chemistry Department, University of Warsaw, Poland

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Abstract

Ultrafine ceria nanoparticles in the size regime of 3–5 nm were synthesized by chemical methods. The oxygen free radical scavenging properties of ceria nanoparticle was studied using electron paramagnetic resonance technique at the concentrations of 1 mM and 10 μ M in the presence of a spin trap. The hydroxyl radical scavenging properties of ceria nanoparticles were found to vary, depending on the concentrations. At lower concentration the catalytic effect was found to be better compared to that at higher concentration, presumably due to the agglomeration at higher concentrations.

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1. Introduction

In living organisms free radicals are produced by a variety of cellular mechanisms [1]. Free radicals are highly reactive and uncontrolled proliferation can lead to alterations of DNA and lipid peroxidation. The oxygen based free radicals in particular, superoxide (O_2^-) and hydroxyl radicals ($\cdot OH$) have been shown to play a vital role in wide variety of clinical diseases including heart attack and cancer [2]. In order to prevent these diseases, it is essential to regulate the presence of oxygen based free radicals [3]. Inorganic nanoparticles (NPs) are shown to be effective in scavenging oxygen free radicals.

Nanoparticles have particle size in the range between 1 and 100 nm and exhibit unique electronic, optical and catalytic properties [4]. Nanoparticles due to their higher

surface area and ability to undergo faster redox reaction can enhance the scavenging of harmful free radicals [5]. Rare earth metal ions such as cerium ions are shown to be an excellent oxygen free radical scavenger and are considered to be less toxic. Cerium oxide (ceria) is a well known component of the modern three-way exhaust-gas catalyst, because it can act as an oxygen reservoir, releasing oxygen under fuel-rich conditions, and absorbing oxygen under deficient conditions. This is achieved through a reversible redox couple with consequent formation/annihilation of surface defects (oxygen vacancies) [6]. Further, the oxide acts as a metal support, stabilizing the dispersion of active noble metal components in these exhaust catalysts [7].

Electron paramagnetic resonance (EPR) spectroscopy is the most direct and definitive technique to monitor the catalytic properties of ceria nanoparticles on radical scavenging process. EPR provides information on the nature and the environment of paramagnetic species having one or more unpaired electrons. Since many oxygen radicals such as superoxide, $\cdot O_2^-$ and hydroxyl radical, $\cdot OH$ have very short life-time, spin-trapping techniques is being used for free radical detection in chemical and biological systems.

* Corresponding author. Fax: +1 407 823 0208.

E-mail address: sseal@mail.ucf.edu (S. Seal).

¹ NSF International REU student.

Spin-trapping utilizes the reaction of unstable free radicals with nitrones or nitroso compounds resulting in the production of long-lived nitroxide radicals that can be detected by EPR spectroscopy. One of the most commonly used spin traps, 5,5-dimethylpyrroline-*N*-oxide (DMPO) reacts with $\cdot\text{O}_2^-$, $\cdot\text{OH}$ forming distinctive spin trap adducts [8,9].

Earlier, we have reported the role of ceria (CeO_2) NPs as radical scavenger in radioprotection [10]. Also, our results showed the effectiveness of ceria NPs in preventing the photoreceptor cell damage due to reactive oxygen species in the primary cell cultures of rat retina [11]. The scavenging property of cerium ions is due to the high thermodynamic affinity for oxygen and the relative ease to undergo reversible redox reaction between Ce^{3+} and Ce^{4+} states [12]. Recently we studied the effect of $\text{Ce}^{3+}/\text{Ce}^{4+}$ ratio in ceria NPs on the superoxide dismutase (SOD) catalytic activity and found higher $\text{Ce}^{3+}/\text{Ce}^{4+}$ ratio resulted in effective catalysis [13]. Various concentrations of nanoparticles ranging from micromolar to nanomolar have been used for *in vitro* and *in vivo* studies [14–16]. Further, in many of the works, the nanoparticle size also varies. As a result, influence concentration on free radical scavenging requires further investigation in order to optimize ceria NPs for safe clinical usage. In the present report we probed the influence of NP concentration on oxygen radical scavenging by electron paramagnetic resonance (EPR) technique.

2. Experimental details

Ceria NPs were synthesized by the reaction of cerium nitrate hexahydrate (Sigma–Aldrich Chemical Inc.) with appropriate amount of hydrogen peroxide. The resultant powder upon drying the solution was analyzed by X-ray diffraction and transmission electron microscopy. Fluorite structured ceria ranges from 3 to 5 nm. The ratio of Ce^{3+} to Ce^{4+} was calculated from X-ray photoelectron spectroscopy. The concentration of Ce^{3+} was 40 at.% as reported earlier [12].

DMPO was used as the spin trap to monitor the changes in hydroxyl radical concentration by EPR spectroscopy. Fenton reaction was used to generate the necessary hydroxyl radicals, prepared by the reaction of iron with hydrogen peroxide. DMPO traps the hydroxyl radicals and forms DMPO-OH adduct. The resultant aminoxyl radical is EPR detectable. To 10 μL of 0.08% H_2O_2 , 16 μL of 0.5 M DMPO, 10 μL of H_2O , 34 μL of 0.1 M phosphate buffer (pH 7.4) were added, followed by 10 μL of 0.1 mM ferrous sulfate [1]. To the above solution 20 μL of ceria NPs with two different concentrations (10 μM and 1 mM) were introduced to study the effect of concentration. The EPR measurements were carried out with a Bruker ELEXSYS E500 instrument operating in the X-band with frequency of 9.4 GHz equipped with a SHQE10115 cavity. In order to elucidate the ceria NP influence, time dependent extinction plots were made in the presence and absence of ceria NPs. All ESR spectra were gathered under room temperature in open air condition, during a single scan.

3. Results and discussion

The EPR spectra for DMPO-OH adduct and simulated spectra are shown in Fig. 1. The spectrum consists of four lines of hyperfine and superhyperfine structure which is related to the interaction of unpaired electron with nitrogen and hydrogen nuclei. In this spectrum the signal coming from one unpaired electron is split into three signals in hyperfine coupling with nitrogen nucleus with spin number $I_{\text{N}} = 1$. Further each of those signals gives rise to two others from superhyperfine interaction with the closest hydrogen (nuclear spin $I_{\text{H}} = 1/2$). These interactions are characterized by two hyperfine parameters a_{N} and a_{H} (14.95 G) which are similar in the case of DMPO-OH adduct ($g = 2.0058$) with the intensity of the spectral lines in the ratio of 1:2:2:1. The observed parameters (a_{N} , a_{H} and g) are in close agreement with those in the literature [15,17–19] indicating the formation of DMPO-OH adduct.

In order to deduce the influence of ceria on the stability of the hydroxyl radical, series of spectra were recorded. The EPR pattern was found to be similar to that of DMPO-OH adduct independent of ceria NP presence with the spectral line intensity of 1:2:2:1. The intensity of the second peak of the spectra was then documented over a period of time for OH radicals in the presence and absence of ceria NPs. Intensities of the hydroxyl spectra and hydroxyl/ceria spectra were plotted versus time in an extinction plot to depict the effects of nanoceria had on the life span of the hydroxyl radicals. In order to estimate the time constants of the radical decay, the intensity plots were fit to the exponential extinction law:

$$I = I_0 + I_1 e^{(-t/t_0)}$$

where I_0 is a constant level of the intensity reached for long time t , I_1 represents the amplitude, and t_0 is the decay constant.

The extinction plots for hydroxyl radical and hydroxyl radical in the presence of 1 mM and 10 μM concentration

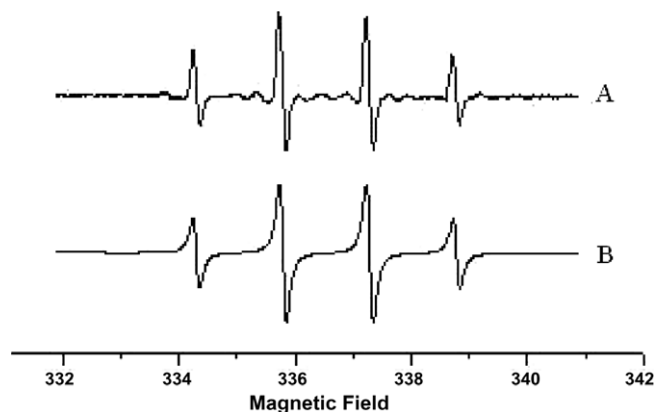


Fig. 1. EPR spectra of DMPO-OH adduct: (A) experimental spectra and (B) simulated spectra.

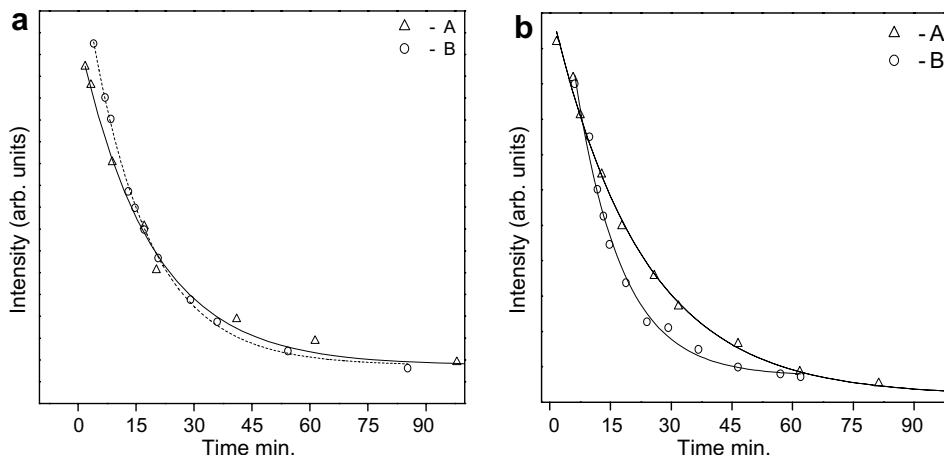


Fig. 2. Extinction plot for ceria nanoparticles with concentrations of (a) 1 mM and (b) 10 μ M, (A) corresponds to the intensity of DMPO-OH adduct in the absence and (B) in the presence of nanoparticles.

of ceria NPs are shown in Fig. 2a and b. The R^2 value upon fitting was found to be 0.98(9) and 0.99(9) for the concentrations of 1 mM (a) and 10 μ M (b), respectively, for nanoceria addition shown in Fig. 2. The extinction plots for both DMPO-OH adduct in the presence and absence of 1 mM ceria NPs are shown in Fig. 2a. The decay constant for the hydroxyl radicals was found to be 18.2 ± 1.4 min and in the presence of 1 mM ceria NPs the decay constant decreased to only about 15.3 ± 0.4 min. Fig. 2b shows the extinction plot for ceria nanoparticles with the concentration of 10 μ M. The intensity of hydroxyl radical decreased drastically in the presence of ceria NPs and saturated to a constant value after about 60 min. The calculated decay constant for hydroxyl radical in the absence of ceria NPs was found to be 19.4 ± 2.1 min which reduced to 7.2 ± 0.2 min in the presence of ceria NPs. Therefore, the addition of ceria at low concentrations seems to have a great effect on the lifespan of the hydroxyl radicals and points to the possibility of using ceria NPs as an effective

free radical scavenger. It is to be noted that size of the ceria NPs was maintained constant in the present experiment.

Recently, photocatalytic activity of ZnO and ceria nanoparticles in forming hydroxyl radical was followed by EPR technique [20,21]. The kinetics of DMPO-OH adduct formation and hence photocatalytic activity was shown to depend on the size ZnO nanoparticles until a critical size. It was shown that ceria nanorods have higher catalytic activity for CO oxidation compared to that of ceria nanoparticles [22]. But, in the present work despite the size of the ceria nanoparticles remaining constant (3–5 nm) still a concentration dependent variation in catalytic activity was observed. This difference can be attributed to the difference in agglomeration behavior as schematically shown in Fig. 3. At higher concentration, the probability to undergo agglomeration for nanoparticles will be relatively higher resulting in a reduction of effective catalytic surface sites available for radical scavenging than that of lower concentrations.

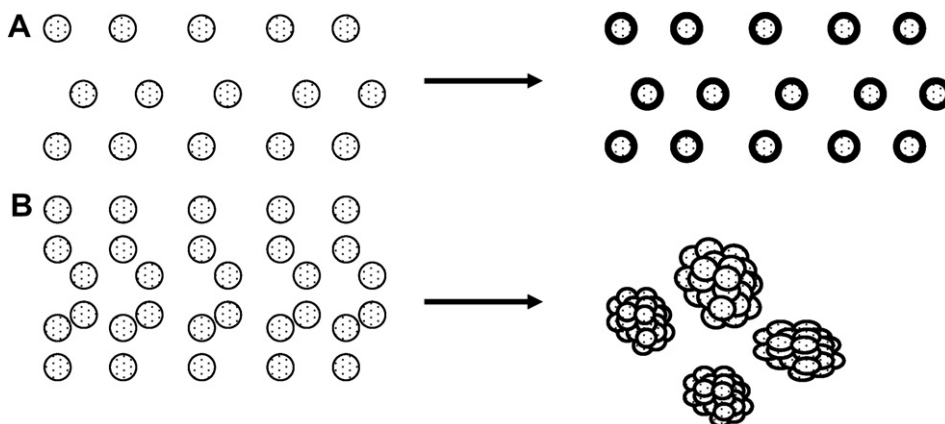


Fig. 3. Lower concentration (A) leads to better catalytic activity of ceria nanoparticles and at higher concentrations (B) agglomeration of nanoparticles reduces the effective catalytic activity.

4. Conclusion

Hydroxyl radicals are harmful to the human body when generated in excess and can be controlled through the use of radical scavengers. In the present work through EPR studies, we have shown that ceria NPs exhibit great promise to be used as a radical scavenger at a lower, optimum concentration. Present experimental result assumes importance as it shows that apart from chemistry, size and morphology, concentrations of nanoparticles also play a vital role in catalysis.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.cplett.2007.06.026](https://doi.org/10.1016/j.cplett.2007.06.026).

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