Antioxidant activity and dose enhancement factor of CeO\textsubscript{2} nanoparticles synthesized by precipitation method

I Nurhasanah\textsuperscript{1}, W Safitri\textsuperscript{1}, Z Arifin\textsuperscript{1}, A Subagio\textsuperscript{1}, and T Windarti\textsuperscript{2}

\textsuperscript{1}Department of Physics, Faculty of Science and Mathematics, Diponegoro University
\textsuperscript{2}Department of Chemistry, Faculty of Science and Mathematics, Diponegoro University, Prof. H. Soedarto St., S.H., Semarang 50275, Indonesia

nurhasanah@fisika.undip.ac.id

Abstract. CeO\textsubscript{2} nanoparticles (CeO\textsubscript{2} NPs) have been considered as promising antioxidant and radioprotectant due to its mixed valence state that can prevent cell damage induced by ionizing radiation. The aim of this study is to investigate the antioxidant activity and dose enhancement factor (DEF) of CeO\textsubscript{2}. The NPs were synthesized by using a precipitation method. The structure of CeO\textsubscript{2} NPs was characterized with an X-ray diffractometer (XRD). The antioxidant activity of CeO\textsubscript{2} NPs was analyzed by the reduction of 1,1-diphenyl-2-picrylhydrazil (DPPH) stable free radical using UV-vis spectroscopy. The dose enhancement factor (DEF) of CeO\textsubscript{2} NPs was examined using X-ray radiation with an energy of 6 MV. The radioprotective ability of CeO\textsubscript{2} NPs was evaluated by mixing CeO\textsubscript{2} NP suspension with \textit{E. coli} and exposing it to X-ray radiation with a dose of 2 Gy. The XRD pattern analysis reveals that CeO\textsubscript{2} NPs possess a cubic fluorite structure. CeO\textsubscript{2} NPs show activity with IC\textsubscript{50} value of 4.4 mg/ml. CeO\textsubscript{2} NPs also show the ability to absorb X-ray radiation with DEF value < 1 and reduce the \textit{E. coli} damage induced by X-ray radiation. The amount of irradiated \textit{E. coli} in the presence of 0.2 mg/ml CeO\textsubscript{2} NPs was found to be 23 times larger than that irradiated \textit{E. coli} in the absence of CeO\textsubscript{2} NPs.

1. Introduction

The application of nanoparticles in radiotherapy has been a subject of considerable interest. Radiotherapy is the reliable way for cancer treatment by exposing cancer cells to ionizing radiation. During radiotherapy of cancer, the ionizing radiation resulted in an increased rate of not only cancer cell death but also normal cell death. The main challenge in the cancer therapy is to increase the radiation ability to kill cancer cells by increasing the dose effectiveness and minimizing damage to the surrounding normal cells. The dose effectiveness to kill cancer cells could be increased by using radiosensitizer, while radioprotector is used to protect normal cell [1-3]. Many free-radical scavengers or antioxidants have been investigated and used to overcome the effects of radiation on normal cell. Amifostine is the only compound as radioprotector recommended by US FDA (Food and Drug Administration) [4, 5]. However, this compound has limitations due to short life and poor penetration to the location where the free radicals produced.

The current nanotechnology makes it possible to develop a variety of nanoparticles to be used for biomedical application with superior properties. Nanoantioxidants, including inorganic nanoparticles have shown promise as high-performance therapeutic nanomedicine in attenuating oxidative stresses. However, the small size and large surface area of nanoparticles resulted in particles aggregation and led
to difficulty in the handling of nanoparticles in liquid [6]. A few metal oxides nanoparticles were reported as antioxidants, including TiO$_2$ [7], ZnO [9-11], and CeO$_2$ [12-17]. Among metal oxide nanoparticles, applications of CeO$_2$ nanoparticles (CeO$_2$ NPs) in biomedical have experienced growing attention due to lower toxicity than the other oxide nanoparticles that commonly investigated such as TiO$_2$ and ZnO. Furthermore, CeO$_2$ nanoparticles are known as catalysts that have pharmacological potential and promising therapeutic agent. The redox reaction that occurs between Ce$^{3+}$ and Ce$^{4+}$ with superoxide and oxidizing hydrogen peroxide are similar to the behavior of antioxidant enzymes [18, 19]. The antioxidant properties of CeO$_2$ NPs are determined by the presence Ce$^{3+}$ which depend on shape, size, valence states and synthesis method [13-15].

This paper describes the antioxidant activity and dose enhancement factor of CeO$_2$ NPs synthesized by precipitation method. The antioxidant activity of nanoparticles CeO$_2$ was examined using DPPH method. The dose enhancement factor of CeO$_2$ NPs was determined using the absorbed dose of X-ray radiation measurement. The capability of CeO$_2$ NPs as radioprotector was evaluated by loading those CeO$_2$ NPs into E. coli culture under X-ray irradiation.

2. Experiment

The CeO$_2$ NPs were synthesized by precipitation method from cerium nitrate solution as described in previous report [20]. The solution of 0.08 M cerium nitrate (Sigma-Aldrich) was prepared in demineralized water/isopropanol mixed solvent with a volume ratio of 1:6. NH$_4$OH was then added drop by drop under stirring into the solution until a pH value of 10 was reached. Precipitates were repeatedly washed by isopropanol. The precipitates were then dried at 60 °C for 2 hours and calcined at 500 °C for 2 hours.

The crystallinity and phase of CeO$_2$ NPs was characterized by X-ray powder diffractometry (XRD) with Cu K$_\alpha$ radiation ($\lambda = 1.54060$ Å). UV-Vis transmittance of the CeO$_2$ NPs was examined using UV-Vis spectrophotometer. Antioxidant activity of CeO$_2$ NPs was evaluated using DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging method. The suspension of CeO$_2$ NPs with different concentration (1.5, 3.0, 4.5, 6.0, 7.5, and 9.0 mg/mL) were sonicated using sonicator bath at room temperature for 10 minutes to avoid agglomeration. The absorbance of samples (the mixture of CeO$_2$ NPs and DPPH solution) and control (DPPH solution without CeO$_2$ NPs) were measured by UV-Vis spectrophotometer. The ability of CeO$_2$ NPs to scavenge DPPH radical was calculated using equation (1). Furthermore, the required concentration to inhibit 50% of DPPH (IC$_{50}$) was determined by linear regression.

$$\% \text{ inhibition} = \frac{A_c - A_s}{A_c} \times 100\%$$

To investigate dose enhancement factor (DEF), the absorbed dose of CeO$_2$ NPs with various mass of 0.5 g, 1 g, 2 g, and 2.5 g was measured using detector. Measurements were made for photon energy of 6 MV and dose rate of 200 cGy/min, SSD of 100 cm, the field size of (2x2) cm$^2$, and detector position at 1 cm below phantom surface. The absorbed dose was measured in the absence and in the presence of CeO$_2$ NPs. The dose enhancement factor (DEF) was defined as ratio of radiation dose in the presence of CeO$_2$ NPs to radiation dose in the absence of CeO$_2$ NPs.

The protection effect of CeO$_2$ NPs was investigated by mixing CeO$_2$ NPs suspension with E. coli culture at various concentrations in the range 0.02 to 0.1 mg/mL. The E. coli cultures were irradiated by using a photon energy clinical linear accelerator (LINAC) of 6 MV X-ray with a dose rate of 200 cGy/min, a depth of 10 cm and source-to-surface distance (SSD) of 90 cm. The number of live E. coli was determined by using total plate count method.

3. Results and discussion

The X-ray diffraction (XRD) pattern of CeO$_2$ NPs synthesized using precipitation method is shown in Figure 1. The XRD pattern reveals that the prepared nanoparticles are polycrystalline of cubic fluoride
CeO\textsubscript{2} structure. Four preferred diffraction peaks at the 2\(\theta\) values of about 28.46°, 32.97°, 47.44° and 56.18° are observed corresponding to (111), (200), (220), and (311) planes, respectively. Those diffraction peaks are in good agreement with the Joint Committee on Powder Diffraction Standard (JCPDS) No. 34-0394. No other peaks related to impurities or other phases are detected in the XRD pattern which confirmed the prepared CeO\textsubscript{2} NPs are single phase crystalline of CeO\textsubscript{2}. This result was confirmed by Rietveld refinement as shown in Figure 1(b). The Rietveld refinement shows good agreement between experimental and simulated XRD pattern with the refinement factors being \(R_{wp} = 9.6\%\) and \(R_p = 7.55\%\) with the goodness-of-fit \(\chi^2 = 1.146\). The refined cell parameter of CeO\textsubscript{2} NPs is 5.419 Å which is slightly larger than that of bulk CeO\textsubscript{2} (5.411 Å) indicating the prepared CeO\textsubscript{2} NPs consist of nanocrystallite. The crystallite size was calculated using Scherer formula to be about 9 nm.

![Figure 1](image1.png)

**Figure 1.** XRD pattern (a) and Rietveld refinement (b) of CeO\textsubscript{2} NPs synthesized by precipitation method.

The UV-Vis absorbance spectrum of the CeO\textsubscript{2} NPs is shown in Figure 2. The peak of absorbance was observed at wavelength of 207 nm and 303 nm corresponding to characteristic absorption peak of Ce\textsuperscript{3+} and Ce\textsuperscript{4+} of CeO\textsubscript{2} NPs. The spectrum reveals that CeO\textsubscript{2} NPs consist of Ce\textsuperscript{3+} and Ce\textsuperscript{4+} ions. The present of Ce\textsuperscript{3+} ion has an important role on the antioxidant activity of CeO\textsubscript{2} NPs [14].

![Figure 2](image2.png)

**Figure 2.** Absorbance spectrum of CeO\textsubscript{2} NPs.

In this study we use DPPH as a stable free radical to determine the radical scavenging activity of CeO\textsubscript{2} NPs. The absorbance of DPPH decreased due to the scavenging of DPPH by donation of electron...
or hydrogen from antioxidant substance to form the stable DPPH molecule. The radical scavenging activity values were expressed as percentage of inhibition as ratio of percentage of sample absorbance decrease and the absorbance of DPPH solution in the absence of antioxidant substance at absorption peak of DPPH ($\lambda = 520$ nm). Figure 3 shows radical scavenging activity of CeO$_2$ NPs. The inhibition of DPPH increased up to 67% at concentration of CeO$_2$ NPs of 9 mg/mL. In another study, CeO$_2$ NPs prepared by co-precipitation method and coated by polysaccharide showed DPPH scavenging activity with % inhibition of 85% [13] whereas, CeO$_2$ NPs prepared by hydrothermal and solvothermal methods showed DPPH scavenging activity up to 55% and 30% respectively [15]. The DPPH scavenging activity of our CeO$_2$ NPs is smaller than levan-coated CeO$_2$ NPs, but higher that that CeO$_2$ NPs prepared by hydrothermal and solvothermal methods. The CeO$_2$ NPs posses antioxidant activity with IC$_{50}$ value of 4.38 mg/ml. The CeO$_2$ NPs exhibited the higher antioxidant activity than ZnO nanoparticles (IC50 ~ 8 – 10 mg/mL) that reported in the literature [9-11]. The antioxidant properties of CeO$_2$ NPs would be explained as due to transfer of electron from CeO$_2$ NPs to odd electron located at nitrogen atom in DPPH.

The protective effect of CeO$_2$ NPs was evaluated to protect E. coli from damage induced by X-ray radiation. Figure 5 demonstrates the protective effect of CeO$_2$ NPs. It can be seen that protective effect depends on concentration of CeO$_2$ NPs. X-ray radiation with dose of 2 Gy killed 98.92% E. coli. The increase in the amount of E. coli was found in E. coli treated with the presence of CeO$_2$ NPs. At the condition with addition of 0.02 mg/mL CeO$_2$ NPs, the amount of E. coli increased by 23 times larger than that irradiated E. coli without addition of CeO$_2$ NPs. These results suggested that CeO$_2$ NPs reduced the amount of E. coli death and protected E. coli damage induced by X-ray radiation. The amount of E. coli death increased with the increase in concentration of CeO$_2$ NPs. The CeO$_2$ NPs can reduce E. coli damage with concentration up to 0.06 mg/mL. However, the addition of CeO$_2$ NPs with concentration larger than 0.06 mg/ml did not act as radioprotector but as radiosensitizer. The protective effect mechanism of CeO$_2$ NPs is physical protection due to its ability to absorb radiation as described by DEF value < 1 and chemical protection through neutralization of reactive oxygen species (ROS) produced by radiolysis of water during irradiation [16]. The main protection mechanism that plays a role in our CeO$_2$ NPs is chemical protection. It is generally attributed to oxygen vacancy corresponding to surface Ce$^{3+}$ fraction and/or Ce$^{3+}$/Ce$^{4+}$ redox switch.

**Figure 3.** Radical scavenging activity of CeO$_2$ NPs.

**Figure 4.** Dose enhancement factor for various mass of CeO$_2$ NPs.
Figure 5. The number of X-ray irradiated E. coli with the presence of CeO$_2$ NPs at various concentration.

4. Conclusion
We have investigated the antioxidant activity and dose enhancement factor of CeO$_2$ NPs synthesized by precipitation method. The CeO$_2$ NPs possess good antioxidant activity and can reduce absorbed dose of X-ray radiation. The CeO$_2$ NPs with concentration of no more than 0.06 mg/ml show a protective effect on E. coli damage induced by X-ray radiation. These results indicated that CeO$_2$ NPs synthesized by precipitation method are a potent radioprotector. However, its effectiveness should be further investigated.

Acknowledgment
This work was financially supported by Ministry of Research, Technology and Higher Education of the Republic of Indonesia, grant No. 007/SP2H/LT/DRPM/IV/2017.

References
[1] Rancoule C Magne N Vallard A Guy J B Rodriguez-Lafrasse C Deutsch E and Chargari C 2016 Cancer Lett. 375 256-62
[2] Retif P Pinel S Toussaïnt M Frochot C Chouikrat R Bastogne T and Barberi-Heyob M 2015 Theranostics 5(9)1030-44
[3] Koukourakis M I 2012 The British Journal of Radiology 85 313-30
[4] Ormsby R J Lawrence M D Blyth B J Bexis K Bezak E Murley J S Grdina D J and Sykes P J 2014 Cell Biol. Toxicol. 30 55-66
[5] Maier P Wenz F and Herskind C 2014 Strahlentherapie und onkologie 190 745-52
[6] Sandhir R Yadav A Sunkaria A and Singhal N 2015 Neurochem. Inter. 89 209-26
[7] Santhoshkumar T Rahuman A A Jayaseelan C Rajakumar G Marimuthu S Kirkthi A V Velayutham K Thomas J Venkatesan J and Kim S K 2014 Asian Pasific J. Tropical Medicine 4(12) 968-76
[8] Nair P M G and Chung I M 2015 Comparative Biochem. and Physiology B 190 1-7
[9] Nethravathi P C Shruthi G S Suresh D Udayabhanu Nagabhushana H and Sharma S C 2015 Ceramic Inter. 41 8680-7
[10] Suresh D Shobharani R M Nethravathi P C Kumar M A P Nagabhushana H and Sharma S C 2015 Spectrochim. Acta Part A 141 128-34
[11] Madan H R Sharma S C Udayabhanu Suresh D Vidya Y S Nagabhushana H Rajanaik H Anantharaju K S Prashanantha S C and Maiya P S 2016 Spectrochim. Acta Part A 152 404-16
[12] Kim S J and Chung B H 2016 Carbohydrate Polymers 150 400-7
[13] Leung Y H Yung M M N Ng A M C Ma A P Y Wong S W Y Chan C M N Ng Y H Djurisic A B Guo M Wong M T Leung F C C Chan W K Leung K M Y and Lee H K 2015 *J. Photochem. Photobiol. B: Biology* **145** 48-59

[14] Dunnick K M Pillai R Pisane K L Stefaniak A B Sabolsky E M and Leonard S S 2015 *Biol. Trace Elem. Res.* **166** 96-107

[15] Soren S Jena S R Samanta L and Parhi P 2015 *Appl. Biochem. Biotechnol.* **177** 148-61

[16] Zholobak N M Shcherbakov A B Bogorad-Kobelska A S Ivanova O S Ye B A Ye S N and Ivanov V K 2014 *J. Photochem. Photobiol. B: Biology* **130** 102-8

[17] Bandyopadhyay S Peralta-Videa J R Plascencia-Villa G Jose-Yacaman M and Gardea-Torresdey J L 2012 *J. Hazard. Mater.* **241-242** 379-86

[18] Walkey C Das S Seal S Erlichman J Heckman V K K Ghibelli L Traversa E McGinnis J F and Self W T 2015 *Environ. Sci. Nano* **2**(1) 33-53

[19] Karakoti A S Monteiro-Riviera N A Aggarwal R Davis J P Narayan R J Self W T McGinnis J F and Seal S 2008 *JOM* **60**(3) 33-7

[20] Nurhasanah I Sutanto H and Futikhaningtyas R 2014 *Adv. Mater. Res.* **896** 108-11