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by Iis Nurhasanah

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The Calcination Temperature Effect on the Antioxidant and Radioprotection Properties of CeO_2 Nanoparticles

Iis Nurhasanah[1,*], Weni Safitri[1], Tri Windarti[2], and Agus Subagio[1]

[1]Department of Physics, Faculty of Science and Mathematics, Diponegoro University
Jl. Prof. Soedarto, SH, Tembalang, Semarang
Telp./Fax. (024) (024)76480822
[2]Department of Chemistry, Faculty of Science and Mathematics, Diponegoro University
Jl. Prof. Soedarto, SH, Tembalang, Semarang

[*]Coresponding author: nurhasanah@fisika.undip.ac.id

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Abstract

The CeO_2 nanoparticles are very interesting to be studied as biomedical materials due to their unique physical and chemical properties. The non-stoichiometric property of CeO_2 plays a role in the redox/catalytic processes that scavenge free radicals. This property has made the potential uses of CeO_2 nanoparticles as antioxidant and radioprotector materials. In this paper, we report the calcination temperature effect on the antioxidant and radioprotective properties of CeO_2 nanoparticles synthesized by precipitation method. The CeO_2 nanoparticles were synthesized at various calcinations temperatures (300-700°C). The formation of CeO_2 nanoparticles and crystallite size was analyzed using X-ray diffractometers. The DPPH method was used to investigate the antioxidant properties of CeO_2. Dose Enhancement Factor (DEF) of CeO_2 nanoparticles were determined by measurement of the absorbed dose of X-ray radiation (Linac 6 MV 200 MU). X-ray diffraction pattern showed formation of cubic fluorite of CeO_2nanoparticles with crystallite size in the range 9 nm-18 nm. Calcination temperature of 500°C resulted in CeO_2 nanoparticles with the best antioxidant properties and lowest DEF value. The radioprotection effect of CeO_2nanoparticles was evaluated based on Escherichia coli survival toward X-ray radiation with a dose of 2 Gy. The CeO_2 nanoparticles increased Escherichia coli survival of about 24.8% order. These results suggested that CeO_2 nanoparticles may potentially be used as radioprotector of X-ray Linac 6 MV.

Keywords: *antioxidant; CeO_2 nanoparticles; Dose Enhancement Factor (DEF); radioprotector*

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INTRODUCTION

Metal oxide nanoparticles have been widely used in various fields of application, i.e.: electronic, energy, environmental remediation and medical. Nowadays, various metal oxide nanoparticles are explored as antioxidant, such as: TiO_2 (Bajic *et al.*, 2017; Santhoshkumar *et al.*, 2014), ZnO (Nethravathi *et al.*, 2015; Suresh *et al.*, 2015; Madan *et al.*, 2016) CuO (Das *et al.*, 2013; Purkayastha *et al.*, 2014), NiO (Madhu *et al.*, 2013; Saikia *et al.*, 2010), SnO_2 (Vidhu and Philip, 2015), MgO (Sushma *et al.*, 2016) and CeO_2 (Kim and Chung, 2016; Dunnick *et al.*, 2015; Soren *et al.*, 2015). Antioxidant is a substance that could delay or prevent oxidation process caused by free radicals.

Antioxidant in a form of nanoparticles (nano-antioxidant) is a new strategy to prevent free radicals caused diseases. In regard to their small size, the nanoparticles antioxidant could interact with biomolecules on the cell surfaces as well as inside the cells. The use of nanoparticles antioxidant was proven to be effective in the prevention of cell damage caused by free radical (Sandhir *et al*., 2015; Liu *et al*., 2008).

CeO_2 nanoparticles are metal oxide nanoparticles, which widely used for medical applications such as radioprotector in the radiotherapy and catalyst for their pharmacological potentials. The combination of strong absorption property to UV light and the interaction of CeO_2 nanoparticles with free radical showed that CeO_2 is a more effective sunscreen material than TiO_2 and ZnO that commonly used in cosmetic products. Moreover, the antioxidant properties of CeO_2 nanoparticles also provide good protection against free radical formed by UV exposure (Zholobak *et al*., 2014; Nurhasanah *et al*., 2014). The ability of CeO_2 nanoparticles to exhibit antioxidant property comes from oxygen vacancy on it surfaces that proceeds redox reaction. Many researches showed that antioxidant properties of CeO_2 were influenced by it shape, size, valence state and synthesis method (Leung *et al*., 2015; Dunnick *et al*., 2015; Soren *et al*., 2015).

CeO_2 nanoparticles also showed high radioprotective activity and health maintenance toward animal living cells (Colon *et al*., 2009). The CeO_2 nanoparticles have been proven could protect healthy tissue around cancer tissue in radiotherapy treatment (Wason and Zao, 2013). However, as found in other synthetic radioprotector materials, the use of CeO_2 is also limited by its toxic concentration limit (dose). The dose will be depended on the radioprotector material structure and the radiated tissue.

In this research, the antioxidant properties and dose enhancement factor of CeO_2 nanoparticles were studied to explore the protection ability of CeO_2 toward radiation from X-rays Linac 6 MV. CeO_2 nanoparticles were synthesized by precipitation method that can be applied at industrial scale. Calcination temperature was varied to study its effect on the antioxidant activity and dose enhancement factor of CeO_2 nanoparticles. The radioprotection ability of CeO_2 nanoparticles was evaluated by using X-rays radiated *Escherichia coli*.

METHOD

CeO_2 nanoparticles were synthesized by precipitation of cerium (III) nitrate hexahydrate (99%, Sigma Aldrich), and followed by calcination process at 300-700°C for 2 hours. The details of the synthesis procedure have been reported previously (Nurhasanah *et al*., 2014). Nanoparticles structure was analyzed using X-rays diffractometer (PW 1710). The nanoparticles antioxidant activity was tested using DPPH method. Dose enhancement factor (DEF) was determined from the measurement of X-rays Linac 6 MV at a dose rate of 200 cGy/minute. Protective effect of CeO_2 nanoparticles with various concentrations (20 to 100 μg) was tested to X-rays radiated *Escherichia coli* (*E. coli*) with a dose of 2 Gy.

RESULTS AND DISCUSSION

The X-ray diffraction pattern of the products synthesized at various calcination temperatures is shown in Figure 1. The diffraction pattern showed crystal plane of (111), (200), (220), (311), (222), (400), (331), (420) and (422) that characteristic to CeO_2 fluorite cubic structure. The sharp and narrow diffraction peaks appear at calcination temperature of 600°C and 700°C, whereas at 300-500°C the peaks have no significant changes with the increasing calcination temperature. The wide diffraction peak indicated that CeO_2 is composed of nano-sized crystallites.

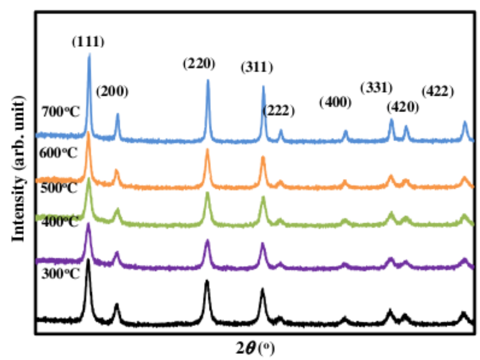

Figure 1. X-rays diffaction pattern of CeO_2 nanoparticles at various calcination temperatures

The crystallites size of CeO_2 nanoparticles was determined using the Scherrer formula expressed by equation (1) for the crystal plane of (111) in Figure 1.

$$D = \frac{k\lambda}{\beta \cos\theta} \quad (1)$$

Where k is a constant of 0.89, β is the full width at half maximum (FWHM) diffraction peak and θ is diffraction angle. Figure 2 shows the crystallites size of CeO_2 nanoparticles at various calcination temperatures. The crystallites size significantly increased at 600°C and 700°C from 9.82 to 17.76 nm. As seen in Figure 1, the peaks for crystal plane of (111) became narrower as calcination temperature increase from 500 to 600 °C and 700°C. The calcination temperature at 300-500°C did not give significant effect on the crystallite size due to insignificant difference of the FWHM. The precipitates still have a high porosity. The calcination at temperature 300 to 500°C has a dominant role in the formation of pores interconnect, so that the crystallite size is almost unchanged. After the interconnect pores are formed, the calcination at temperature > 500°C contributes to the crystallite growth.

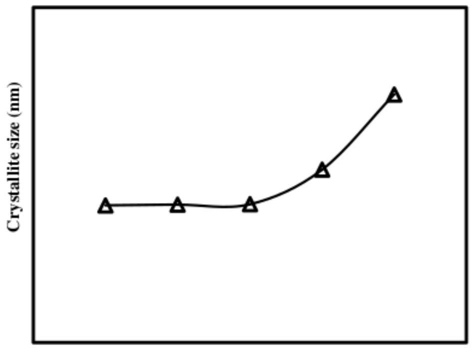

Figure 2. Crystallite size of CeO_2 nanoparticles at various calcination temperatures

Antioxidants are compounds that can prevent oxidation by free radicals. Antioxidants react with free radicals so they become unreactive, stabilizing the free radicals by pairing the electrons and prevent chain reactions. The antioxidant activity of the CeO_2 nanoparticles was tested by DPPH method for its simplicity and high accuracy. The antioxidant activity is expressed as the value of IC_{50} which reflects the amount of antioxidant compounds required to reduce 50% of DPPH absorbance.

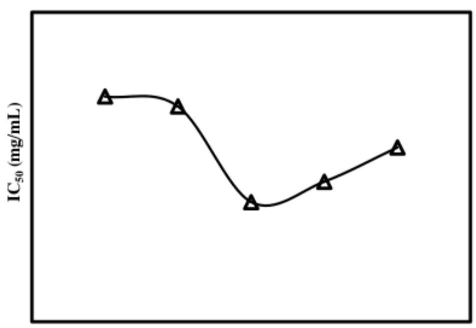

Figure 3. Antioxidant activity of CeO_2 nanoparticles at various calcination temperatures

Figure 3 shows the calculated IC_{50}value of CeO_2 nanoparticles obtained at various temperatures. The IC_{50} values were ranged from 4.38 to 4.73 mg/mL. The smallest IC_{50} value indicates the highest antioxidant activity. The data showed that calcination at 500°C produced CeO_2 nanoparticles with the highest antioxidant activity. The antioxidant activity of CeO_2 nanoparticles come from Ce^{3+} valency state and oxygen vacancy. Electrons were transfered from CeO_2 to nonpaired electron of nitrogen in DPPH (Zholobak *et al*., 2014; Dunnick *et al*., 2015). Furthermore, antioxidant activity of CeO_2nanoparticles may also be affected by the size, shape and synthesis method. The antioxidant activity of CeO_2 nanoparticles obtained in this research is higher than those synthesized using solvothermal method that only prevent 30% of DPPH free radicals (Soren *et al*., 2015). It is also higher than ZnO nanoparticles that have IC_{50} value about 8 mg/mL (Nethravathi *et al*., 2015; Suresh *et al*., 2015; Madan *et al*., 2016).

Antioxidant compounds are used in cancer therapy to protect normal cells from radiation effects. The interaction of radiation with the cells will produce free radical that derived from radiolysis reactions. Then the free radicals will react with cancer cells and also with normal cells around the cancer. To minimize the radiation effect on the normal cells, radioprotector compounds are required. They should be able to protect normal cells from radiation damage. A compound has potential as radioprotector if it could absorb (decrease) radiation dose or has dose enhancement factor (DEF) less than one. Figure 4 shows DEF value of CeO_2 nanoparticles that calcined at various temperatures for X-rays LINAC 6 MV at a dose of 2 Gy. The DEF of CeO_2 nanoparticles is ranging from 0.9918 to 0.9934, which is smaller than one. This indicates the potential of CeO_2 nanoparticles as a radioprotector. The CeO_2 nanoparticles synthesized at 500°C possess the lowest DEF value. This result is consistent with the antioxidant activity as shown in Figure 3.

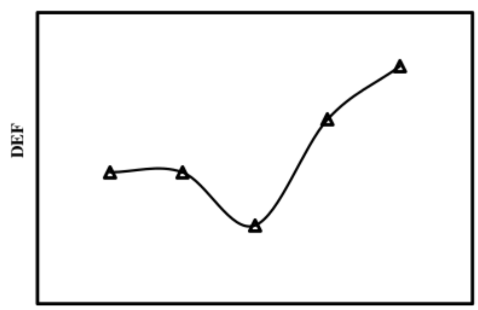

Figure 4. DEF of CeO_2 nanoparticles at various calcination temperatures

The potential of CeO_2 nanoparticles as a radioprotector was further tested using *E. coli* bacteria that radiated with X-rays Linac 6 MV at a dose of 2 Gy. The CeO_2 nanoparticles were added to *E. coli* media with various concentrations to determine the effectiveness of CeO_2 nanoparticles as radioprotector. The ability of nanoparticles to protect *E. coli* from damage that caused by radiation is indicated by the percentage of *E. coli* survival as shown in Figure 5.

The addition of CeO_2 nanoparticles to radiated *E. coli* has improved its survival percentage. Treatments of radiated *E. coli* with 20; 40 and 60 μg of CeO_2 nanoparticles have increased the survival percentage as 24.8, 14.8 and 12.28%, which are higher than the untreated one. The data showed that addition of 20 μg CeO_2 nanoparticles exhibited the highest survival percentage of *E. coli* due to their ability in reducing the cells damage. The survival percentage of bacteria was reduced as the CeO_2 nanoparticles concentration increased. It can be said that CeO_2 nanoparticles have an optimum concentration when they are used as a radioprotector. The addition of nanoparticles from 20 to 60 μg provides a protective effect against radiation.

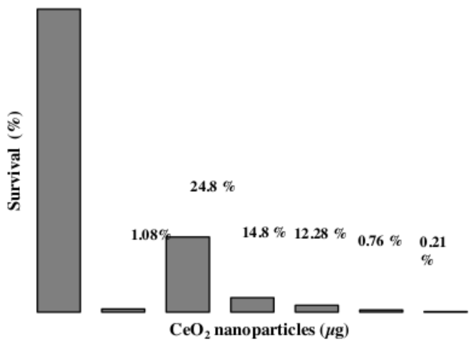

Figure 5. Survival precentage of *E. coli* after irrradiated by X-ray and treated with CeO_2 nanoparticles

In general, there are three mechanisms that could cause the death of bacteria; (i) incident or primary radiation, (ii) interaction between *E. coli* and secondary electrons, and (iii) interaction of *E. coli* with free radicals. Secondary electrons are produced from the interactions of incident radiation with CeO_2 nanoparticles. Meanwhile, the free radicals are generated from the interactions between H_2O and O_2 molecules with incident radiation and interactions of H_2O and O_2 with secondary electrons (Colon *et al*., 2009).

$$H_2O \xrightarrow{\text{irradiation}} H_2O^+ + e^- \rightarrow OH^* + H_3O^+ + e^-_{aq}$$

The additions of CeO_2 nanoparticles at a certain concentration lead the free radicals to interact with the charge present on the CeO_2 nanoparticles surface and to pair with. In the nanoscale dimension, CeO_2 nanoparticles possess two valences state Ce^{3+} and Ce^{4+} (*co-exist*) present on its surface. The CeO_2 can reversibly oxidized from Ce^{3+} and Ce^{4+} through interaction with the free radicals as described by following reactions (Karakoti *et al*., 2008).

$$Ce^{4+} + e^- \leftrightarrow Ce^{3+}$$
$$Ce^{3+} + OH^* \rightarrow Ce^{4+} + OH^-$$
$$Ce^{4+} + O_2^{*-} \leftrightarrow Ce^{3+} + O_2$$

Based on this research, the radioprotective activity of CeO_2 nanoparticles can be increased by reducing the concentration of CeO_2 nanoparticles.

CONCLUSION

CeO_2 nanoparticles synthesized using precipitation method at calcination temperature of 300-700^{o}C showed antioxidant activity with IC_{50} of 4.38 to 4.73 mg/mL. Dose Enhancement Factor of CeO_2 nanoparticles were less than one, ranging from 0.9918-0.9934. The Addition of CeO_2 nanoparticles with concentration of 20μg reduced the damage cells induced by X-ray radiation and improved *E. coli* survival by 24.8%. CeO_2 nanoparticles can be recommended as X-rays LINAC 6 MV radioprotector. Further research is needed to obtain the optimum radioprotective activity of CeO_2 nanoparticles.

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