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HASIL PENILAIAN SEJAWAT SEBIDANG ATAU PEER REVIEW
KARYA ILMIAH: JURNAL ILMIAH**

Judul Jurnal Ilmiah (Artikel) : The Calcination Temperature Effect On The Antioxidant And Radioprotection Properties Of CeO₂ Nanoparticles

Penulis Jurnal Ilmiah/Jumlah Penulis : Iis Nurhasanah, Weni Safitri, Tri Windarti, Agus Subagio/ 4 orang

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p-ISSN 0852-0798

c. Vol, No., Bln Thn : Vol. 18, No. 1, Maret 2018

d. Penerbit : Diponegoro University

e. DOI (jika ada) : 10.14710/reaktor.18.1.22-26

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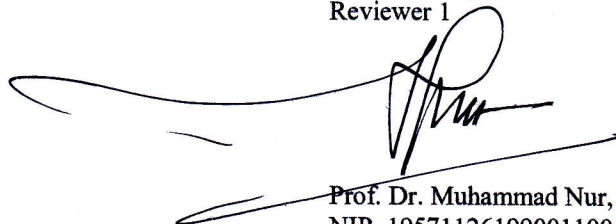
Reviewer II



Prof. Dr. Wahyu Setiabudi, M.S
NIP. 195806151985031002
Unit Kerja: Departemen Fisika, Fakultas Sains dan Matematika UNDIP Semarang

Semarang, April 2020 3

Reviewer I



Prof. Dr. Muhammad Nur, DEA
NIP. 195711261990011001
Unit Kerja: Departemen Fisika, Fakultas Sains dan Matematika UNDIP Semarang

= 3,29

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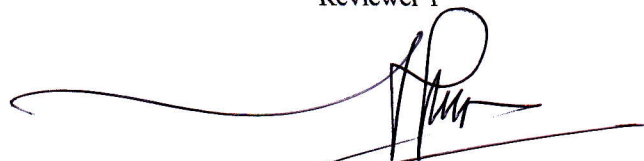
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Reviewer 1



Prof. Dr. Muhammad Nur, DEA
NIP. 195711261990011001

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d. Kelengkapan unsur dan kualitas penerbit (30%)		7,5		7,5
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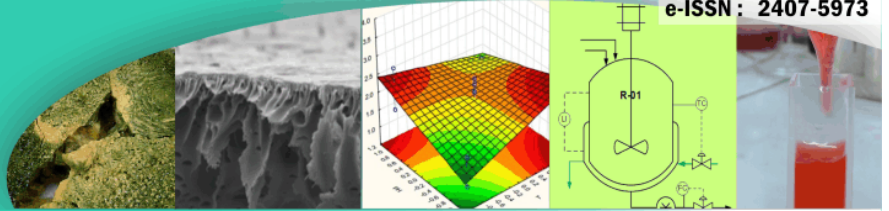
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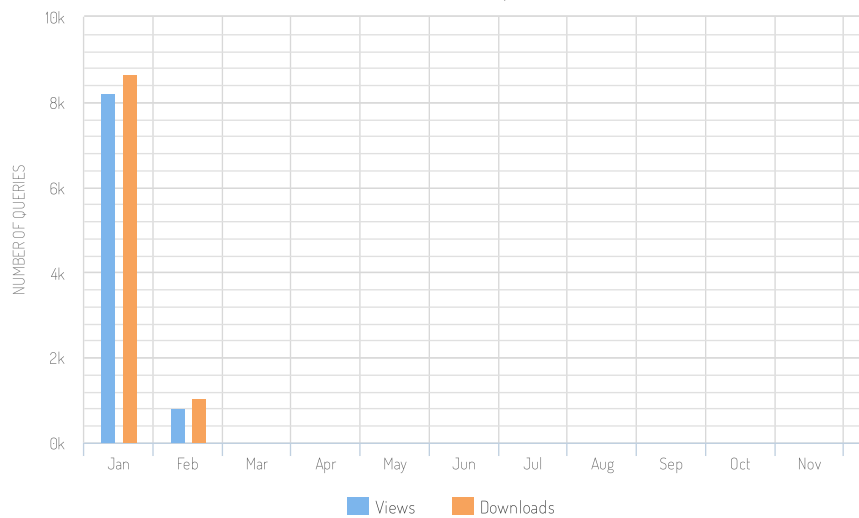
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<u>Pengaruh proses pengeringan rimpang temulawak (<i>Curcuma xanthorrhiza roxb</i>) terhadap kandungan dan komposisi kurkuminoid</u> B Cahyono, MDK Huda, L Limantara Reaktor 13 (3), 165-171	41
<u>Characterisation of solid and liquid pineapple waste</u> A Abdullah, H Mat Reaktor 12 (1), 48-52	38
<u>Pembuatan kitosan dari limbah cangkang udang serta aplikasinya dalam mereduksi kolesterol lemak kambing</u> H Hargono, A Abdullah, I Sumantri Reaktor 12 (1), 53-57	37
<u>Karakteristik fisikokimia pati sagu hasil pengeringan secara fluidisasi menggunakan alat pengering cross flow fluidized bed bertenaga surya dan biomassa</u> A Jading, E Tethool, P Payung, S Gultom Reaktor 13 (3), 155-164	35
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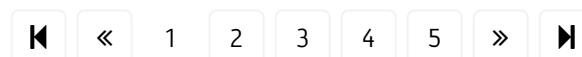
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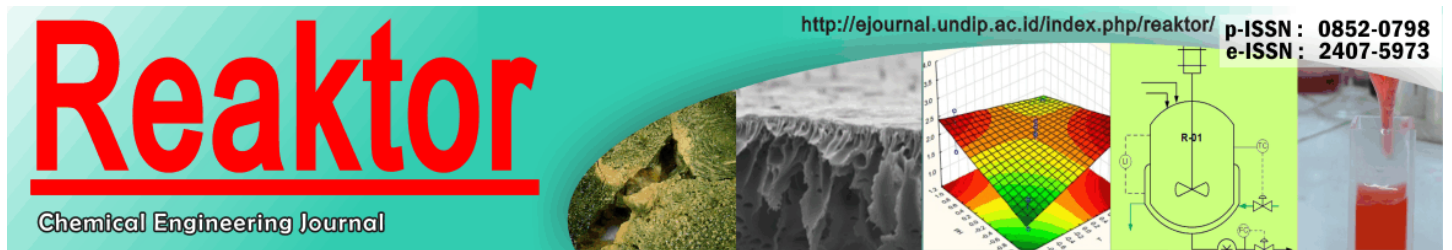


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THE CALCINATION TEMPERATURE EFFECT ON THE ANTIOXIDANT AND RADIOPROTECTION PROPERTIES OF CeO₂ NANOPARTICLES

by Agus Subagio

Submission date: 06-Feb-2020 03:06PM (UTC+0700)

Submission ID: 1252468953

File name: mperature_Effect_On_The_Antioxidant_And_Radioprotection_Prop.pdf (638.01K)

Word count: 2980

Character count: 17059

The Calcination Temperature Effect on the Antioxidant and Radioprotection Properties of CeO₂ Nanoparticles

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(Received: November 1, 2017 ; Accepted: February 28, 2018)

Abstract

The CeO₂ nanoparticles are very interesting to be studied as biomedical materials due to their unique physical and chemical properties. The non-stoichiometric property of CeO₂ plays a role in the redox/catalytic processes that scavenge free radicals. This property has made the potential uses of CeO₂ nanoparticles as antioxidant and radioprotector materials. In this paper, we report the calcination temperature effect on the antioxidant and radioprotective properties of CeO₂ nanoparticles synthesized by precipitation method. The CeO₂ nanoparticles were synthesized at various calcinations temperatures (300-700°C). The formation of CeO₂ nanoparticles and crystallite size was analyzed using X-ray diffractometers. The DPPH method was used to investigate the antioxidant properties of CeO₂. Dose Enhancement Factor (DEF) of CeO₂ 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 CeO₂ nanoparticles with crystallite size in the range 9 nm-18 nm. Calcination temperature of 500°C resulted in CeO₂ nanoparticles with the best antioxidant properties and lowest DEF value. The radioprotection effect of CeO₂ nanoparticles was evaluated based on *Escherichia coli* survival toward X-ray radiation with a dose of 2 Gy. The CeO₂ nanoparticles increased *Escherichia coli* survival of about 24.8% order. These results suggested that CeO₂ nanoparticles may potentially be used as radioprotector of X-ray Linac 6 MV.

Keywords: antioxidant; CeO₂ nanoparticles; Dose Enhancement Factor (DEF); radioprotector

How to Cite This Article: Nurhasanah, I., Safitri, W., Windarti, T., and Subagio, A., (2018), The Calcination Temperature Effect on the Antioxidant and Radioprotection Properties of CeO₂ Nanoparticles, Reaktor, 18(1), 22-26, <http://dx.doi.org/10.14710/reaktor.18.1.22-26>

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: TiO₂ (Bajic *et al.*, 2017; Santhoshkumar *et al.*, 2014), ZnO (Nethravathi

¹*et al.*, 2015; Suresh *et al.*, 2015; Man *et al.*, 2016) CuO (Das *et al.*, 2013; Purkayastha *et al.*, 2014), NiO (Madhu *et al.*, 2013; Saikia *et al.*, 2010), SnO₂ (Vidhu and Philip, 2015), MgO (Sushma *et al.*, 2016) and CeO₂ (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).

CeO₂ 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 CeO₂ nanoparticles with free radical showed that CeO₂ is a more effective sunscreen material than TiO₂ and ZnO that commonly used in cosmetic products. Moreover, the antioxidant properties of CeO₂ nanoparticles also provide good protection against free radical formed by UV exposure (Zholobak *et al.*, 2014; Nurhasanah *et al.*, 2014). The ability of CeO₂ nanoparticles to exhibit antioxidant property comes from oxygen vacancy on its surfaces that proceeds redox reaction. Many researches showed that antioxidant properties of CeO₂ were influenced by its shape, size, valence state and synthesis method (Leung *et al.*, 2015; Dunnick *et al.*, 2015; Soren *et al.*, 2015).

CeO₂ nanoparticles also showed high radioprotective activity and health maintenance toward animal living cells (Colon *et al.*, 2009). The CeO₂ 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 CeO₂ 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 CeO₂ nanoparticles were studied to explore the protection ability of CeO₂ toward radiation from X-rays Linac 6 MV. CeO₂ 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 CeO₂ nanoparticles. The radioprotection ability of CeO₂ nanoparticles was evaluated by using X-rays radiated *Escherichia coli*.

METHOD

CeO₂ 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 CeO₂ 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 CeO₂ 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 CeO₂ is composed of nano-sized crystallites.

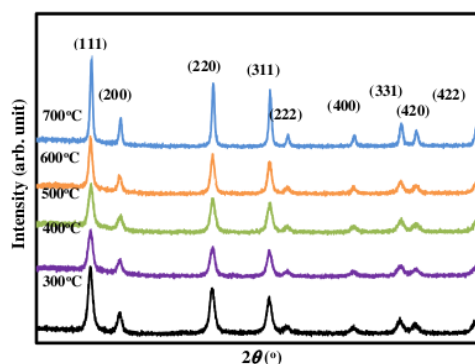


Figure 1. X-rays diffraction pattern of CeO₂ nanoparticles at various calcination temperatures

The crystallites size of CeO₂ 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 CeO₂ 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.

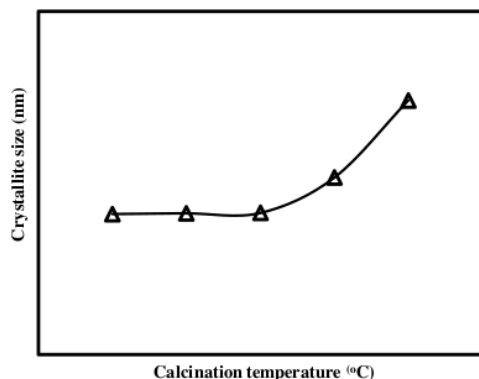


Figure 2. Crystallite size of CeO₂ nanoparticles at various calcination temperatures

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 CeO₂ nanoparticles was tested by DPPH method for its simplicity and high accuracy. The antioxidant activity is expressed as the value of IC₅₀ which reflects the amount of antioxidant compounds required to reduce 50% of DPPH absorbance.

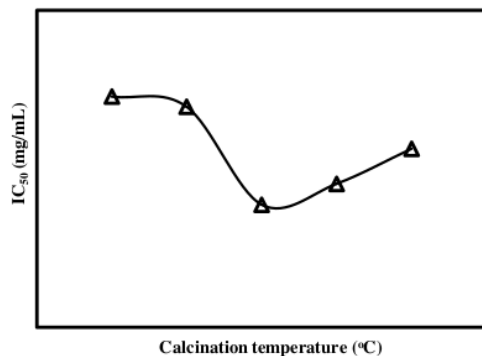


Figure 3. Antioxidant activity of CeO₂ nanoparticles at various calcination temperatures

Figure 3 shows the calculated IC₅₀ value of CeO₂ nanoparticles obtained at various temperatures. The IC₅₀ values were ranged from 4.38 to 4.73 mg/mL. The smallest IC₅₀ value indicates the highest antioxidant activity. The data showed that calcination at 500°C produced CeO₂ nanoparticles with the highest antioxidant activity. The antioxidant activity of CeO₂ nanoparticles come from Ce³⁺ valency state and oxygen vacancy. Electrons were transferred from CeO₂ to nonpaired electron of nitrogen in DPPH (Zholobak *et al.*, 2014; Dunnick *et al.*, 2015). Furthermore, antioxidant activity of CeO₂ nanoparticles may also be affected by the size, shape and synthesis method. The antioxidant activity of CeO₂ 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 IC₅₀ 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 CeO₂ nanoparticles that calcined at various temperatures for X-rays LINAC 6 MV at a dose of 2 Gy. The DEF of CeO₂ nanoparticles is ranging from 0.9918 to 0.9934, which is smaller than one. This indicates the potential of CeO₂ nanoparticles as a radioprotector. The CeO₂ nanoparticles synthesized at 500°C possess the lowest DEF value. This result is consistent with the antioxidant activity as shown in Figure 3.

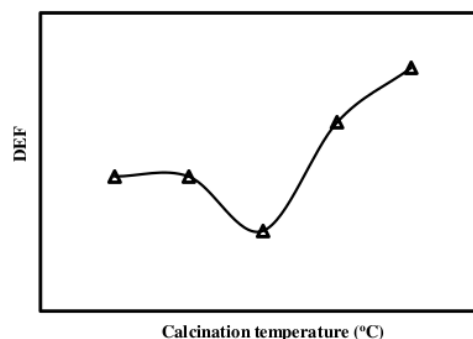


Figure 4. DEF of CeO₂ nanoparticles at various calcination temperatures

The potential of CeO₂ 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 CeO₂ nanoparticles were added to *E. coli* media with various concentrations to determine the effectiveness of CeO₂ 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 CeO₂ nanoparticles to radiated *E. coli* has improved its survival percentage. Treatments of radiated *E. coli* with 20; 40 and 60 µg of CeO₂ 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 CeO₂ 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 CeO₂ nanoparticles concentration increased. It can be said that CeO₂ 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.

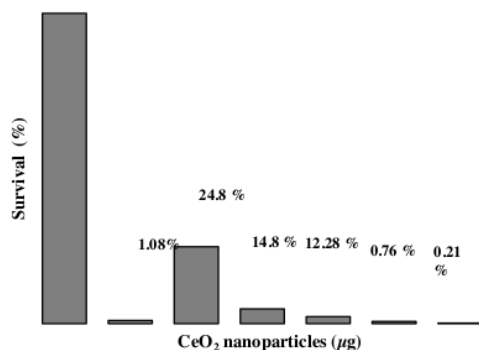
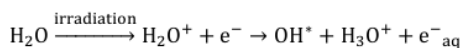
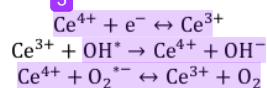


Figure 5. Survival percentage of *E. coli* after irradiated by X-ray and treated with CeO₂ nanoparticles

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 CeO₂ nanoparticles. Meanwhile, the free radicals are generated from the interactions between H₂O and O₂ molecules with incident radiation and interactions of H₂O and O₂ with secondary electrons (Colon *et al.*, 2009).



The additions of CeO₂ nanoparticles at a certain concentration lead the free radicals to interact with the charge present on the CeO₂ nanoparticles surface and to pair with. In the nanoscale dimension, CeO₂ nanoparticles possess two valences state Ce³⁺ and Ce⁴⁺ (*co-exist*) present on its surface. The CeO₂ can reversibly oxidized from Ce³⁺ and Ce⁴⁺ through interaction with the free radicals as described by following reactions (Karakoti *et al.*, 2008).



Based on this research, the radioprotective activity of CeO₂ nanoparticles can be increased by reducing the concentration of CeO₂ nanoparticles.

CONCLUSION

CeO₂ nanoparticles synthesized using precipitation method at calcination temperature of 300-700°C showed antioxidant activity with IC₅₀ of 4.38 to

4.73 mg/mL. Dose Enhancement Factor of CeO₂ nanoparticles were less than one, ranging from 0.9918-0.9934. The Addition of CeO₂ nanoparticles with concentration of 20 μg reduced the damage cells induced by X-ray radiation and improved *E. coli* survival by 24.8%. CeO₂ nanoparticles can be recommended as X-rays LINAC 6 MV radioprotector. Further research is needed to obtain the optimum radioprotective activity of CeO₂ nanoparticles.

ACKNOWLEDGEMENTS

This research was funded by Ministry of Research, Technology and Higher Education with contract number: No. 007/SP2H/ LT/DRPM/IV/2017.

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