Effect of Gd substitution on the crystal structure, magnetic susceptibility and biocompatibility of nano-sized Ca$_{10-x}$Gd$_x$(PO$_4$)$_6$(OH)$_2$ particles

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Abstract. This research investigated the crystal structure, magnetic susceptibility, and biocompatibility of the nano-sized Ca$_{10-x}$Gd$_x$(PO$_4$)$_6$(OH)$_2$ particles for $x =$ 0%, 1.00%, 1.25%, 1.50%, and 1.75%. The hydrolysis method was employed to produce the nano-sized Ca$_{10-x}$Gd$_x$(PO$_4$)$_6$(OH)$_2$ particles from natural calcite. The crystal structure and magnetic susceptibility of the nano-sized Ca$_{10-x}$Gd$_x$(PO$_4$)$_6$(OH)$_2$ particles were characterized by means of X-ray diffraction (XRD) and susceptibility meter, respectively. The elemental data analysis presented that the natural calcite as the main precursor contained calcium of 98.84%. Moreover, the XRD data showed that the increase in Gd content decreased the lattice parameters and crystal volume of the nano-sized Ca$_{10-x}$Gd$_x$(PO$_4$)$_6$(OH)$_2$ particles. The magnetic character of the nano-sized Ca$_{10-x}$Gd$_x$(PO$_4$)$_6$(OH)$_2$ particles changed from diamagnetic to paramagnetic as the increase of Gd content. Interestingly, the increasing Gd content enhanced the magnetic susceptibility of the nano-sized Ca$_{10-x}$Gd$_x$(PO$_4$)$_6$(OH)$_2$ particles. Furthermore, the nano-sized Ca$_{10-x}$Gd$_x$(PO$_4$)$_6$(OH)$_2$ particles had an excellent biocompatibility. Therefore, the prepared nano-sized Ca$_{10-x}$Gd$_x$(PO$_4$)$_6$(OH)$_2$ particles open a potency to be applied for biomedical treatment.

1. Introduction
Over the past five years, hydroxyapatite/HA (Ca$_x$(PO$_y$)(OH)$_z$) nanoparticles has attracted many researchers in various biomedical fields [1], [2], [3], [4], [5], [6] due to their superior performances such as biocompatibility, bioactivity, non-toxic, bio-absorbability, and osteo-conductivity [7], [8], [9], [10]. Notably, several studies presented that the nano-sized Ca$_x$(PO$_y$)(OH)$_z$ particles had been used as biomedical materials, for example for bone replacement [11], scaffolds [12], [13], and drug delivery vehicle [14], [15]. Regarding the application development of the nano-sized Ca$_x$(PO$_y$)(OH)$_z$ particles, particle size and phase purity become essential role to consider. As an illustration, the nano-sized Ca$_x$(PO$_y$)(OH)$_z$ particles with high phase purity had excellent performance in improving the adsorption in tissues [16] and as drug delivery systems [17].

In order to be used in biomedical applications related to magnetic properties, the nano-sized Ca$_x$(PO$_y$)(OH)$_z$ particles have limitation due to their diamagnetic character [18]. Therefore, to overcome such problem, several efforts need to be explored such as by enhancing the magnetic
properties of the nano-sized Ca$_x$(PO$_4$)$_2$(OH)$_2$ particles. One of the effective ways is by modifying Ca atom in the nano-sized Ca$_x$(PO$_4$)$_2$(OH)$_2$ particles with other metals or metal oxides. A previous study reported that modification of the nano-sized Ca$_x$(PO$_4$)$_2$(OH)$_2$ particles by ZnO was successfully conducted for osteoblast mineralization [19]. Furthermore, another study also reported that TiO$_2$-doped Ca$_x$(PO$_4$)$_2$(OH)$_2$ particles could improve the ability of load-bearing implant without reducing its size [20]. However, such studies could not enhance the magnetic properties of the nano-sized Ca$_x$(PO$_4$)$_2$(OH)$_2$ particles. Hence, substitution should be carried out by selected an appropriate atom such as gadolinium (Gd).

In particular, the Gd substitution to replace Ca in the nano-sized Ca$_x$(PO$_4$)$_2$(OH)$_2$ particles can be conducted to form Ca$_x$Gd$_y$(PO$_4$)$_2$(OH)$_2$ system. Theoretically, Gd is lanthanide group element in [Xe] 4f $^7$ 5d $^6$ 6s configuration which has big magnetic moment due to the unpaired electrons. If an external magnetic field is applied, it causes macroscopic magnetic field and affects the surrounding nucleus. Based on the recent study, as a contrast agent, Gd oxide in the form of Gd$^3+$ ion could clarify the visualization by enhancing positive contrast level [21]. In order to be applied in some biomedical applications, the fundamental investigation such as crystal structure, magnetic susceptibility, and biocompatibility become important to be conducted. Furthermore, to reduce the fabrication cost, we explored the natural resource from natural calcite that easily found in Indonesia.

2. Methods

The nano-sized Ca$_x$(PO$_4$)$_2$(OH)$_2$ particles were prepared through several steps. The first step was initial preparation of natural calcite as the main Ca source. The second step was synthesis of the nano-sized Ca$_x$(PO$_4$)$_2$(OH)$_2$ particles started by reacting Ca(OH)$_2$ and Gd powders with HNO$_3$ to form Ca(NO$_3$)$_2$. The Ca(NO$_3$)$_2$ was then reacted with (NH$_4$)$_2$HPO$_4$ and NH$_2$OH via stirring process for 2 h and controlling pH in a base condition (pH ~ 9-10). The last product was then precipitated for 24 h and continuously followed by washing process using H$_2$O. The Ca$_x$Gd$_y$(PO$_4$)$_2$(OH)$_2$ powders were then obtained after drying process at for 1 h. The composition of the Gd was maintained with the values of $x = 0\%$, 1.00%, 1.25%, 1.50%, and 1.75%. The Ca$_x$Gd$_y$(PO$_4$)$_2$(OH)$_2$ nanoparticles were characterized by means of X-ray diffraction (XRD) and susceptibility meter to investigate their respective crystal structure and magnetic susceptibility. The biocompatibility character of the prepared samples was investigated based on toxicity of the nano-sized Ca$_x$(PO$_4$)$_2$(OH)$_2$ particles by recording the intestinal contraction of the animal research.

3. Results and Discussion

Experimental data of the elemental mapping showed that the natural calcite used as a calcium source for Ca$_x$Gd$_y$(PO$_4$)$_2$(OH)$_2$ synthesis contained Ca of 98.84%. In the natural calcite, there were a relatively few impurity elements such as Fe (0.05%), Co (0.084%), Cu (0.04%), Er (0.10%), and Yb (0.76%). Furthermore, the X-ray diffraction patterns of the Ca$_x$Gd$_y$(PO$_4$)$_2$(OH)$_2$ particles which is presented in Figure 1 shows the diffraction patterns of each sample directing upward ($\theta$ is getting higher) is getting higher Gd composition with $x = 0$ as Ca$_x$(PO$_4$)$_2$(OH)$_2$ particles. The diffraction peak shifted to higher position of 2\theta at the main peak. This pattern indicated that Gd$^3+$ ion with doping 0.0100 $\leq$ $x$ $\leq$ 0.0175 had been inserted into Ca$_x$(PO$_4$)$_2$(OH)$_2$, substituting a half of Ca$^{2+}$ ions. The wide diffraction peaks were also observed in all samples meaning that the samples had nanometric size. The quantitative data analysis informed that the crystallite sizes of the samples were 15.85, 9.40, 9.34, 9.03, and 6.95 nm, respectively for $x = 0\%$, 1.00%, 1.25%, 1.50%, and 1.75%. The results of quantitative analysis of the nano-sized Ca$_x$(PO$_4$)$_2$(OH)$_2$ particles are presented in Table 1.
Table 1. Results of data analysis for the nano-sized Ca\textsubscript{10}(PO\textsubscript{4})\textsubscript{6}(OH)\textsubscript{2} particles

| \(x\)   | 0%  | 1.00% | 1.25% | 1.50% | 1.75% |
|---------|-----|-------|-------|-------|-------|
| \(a = b\) (Å) | 9.453 | 9.394 | 9.389 | 9.388 | 9.341 |
| \(c\) (Å) | 6.878 | 6.868 | 6.852 | 6.853 | 6.847 |
| \(V\) (Å\(^3\)) | 532.32 | 524.93 | 523.11 | 523.06 | 517.37 |
| Particle size (nm) | 15.85 | 9.40 | 9.34 | 9.03 | 6.95 |
| GoF (%) | 1.4 | 2.1 | 2.2 | 1.9 | 2.1 |
| Rwp | 25.1 | 30.8 | 31.4 | 30.9 | 29.8 |
| Rp | 19.2 | 23.4 | 25.1 | 23.9 | 22.8 |
| Rexp | 21.2 | 20.9 | 21.1 | 22.2 | 20.5 |

Figure 1. X-ray diffraction patterns of the nano-sized Ca\textsubscript{10}(PO\textsubscript{4})\textsubscript{6}(OH)\textsubscript{2} particles

Figure 2. Three dimensional structure of Ca\textsubscript{10-x}Gd\textsubscript{x}(PO\textsubscript{4})\textsubscript{6}(OH)\textsubscript{2} particles

Based on Table 1, the Ca\textsubscript{10-x}Gd\textsubscript{x}(PO\textsubscript{4})\textsubscript{6}(OH)\textsubscript{2} particles crystallized with hexagonal structure [22]. The lattice parameters values of \(a = b\) were in the range of 9.341 – 9.453 Å and the values \(c\) were in the range of 6.847 – 6.878 Å. At a glance, all lattice parameters decreased as the increase in Gd\textsuperscript{3+} ion...
composition inserted into Ca\(^{2+}\) ion that reduced the lattice parameter and crystal volume. This case means that if Gd was doped on the hexagonal area occupied by Ca ion, some of Ca ions would be replaced by Gd ions based on doping composition. The decrease in lattice parameter was because the Gd\(^{3+}\) radius was smaller than Ca\(^{2+}\) radius (the Gd\(^{3+}\) ion radius = 1.00 Å and Ca\(^{2+}\) ion radius = 1.04 Å). In line with those work, Madhumathi et al. also reported that doping of Gd\(^{3+}\) ion into the nano-sized Ca\(_{10}\)(PO\(_4\))\(_6\)(OH\(_2\)) particles reduced their lattice parameter due to the difference in ion radius [23].

Table 2. Magnetic susceptibility of the nano-sized Ca\(_{10}\)(PO\(_4\))\(_6\)(OH\(_2\)) particles

| \(x\) | Susceptibility (emu/g Oe) |
|------|--------------------------|
| 0%   | -0.0018\(\times10^{-6}\) |
| 1.00%| 0.0014\(\times10^{-6}\)  |
| 1.25%| 0.0015\(\times10^{-6}\)  |
| 1.50%| 0.0028\(\times10^{-6}\)  |
| 1.75%| 0.0031\(\times10^{-6}\)  |

The magnetic susceptibility values of the nano-sized Ca\(_{10}\)(PO\(_4\))\(_6\)(OH\(_2\)) particles are presented in Table 2. Based on the data analysis, the susceptibility values of the nano-sized Ca\(_{10}\)(PO\(_4\))\(_6\)(OH\(_2\)) particles increased as the increase in Gd\(^{3+}\) dopant composition. This physical performance showed that the contribution of Gd\(^{3+}\) ion substitution into Ca\(_{10}\)(PO\(_4\))\(_6\)(OH\(_2\)) in forming Ca\(_x\)Gd\(_{1-x}\)(PO\(_4\))\(_6\)(OH\(_2\)) structure could improve susceptibility of material to be more paramagnetic than before which was diamagnetic. Meanwhile, the similar research results were reported by Chen et al. stating that magnetization level of Ca\(_{10}\)Gd\(_{1-x}\)(PO\(_4\))\(_6\)(OH\(_2\)) nanoparticles increased as the increase in dopant composition [24]. Nevertheless, that study could not show the susceptibility value of material. In another side, when Gd\(^{3+}\) ion had not been inserted into Ca\(_{10}\)Gd\(_{1-x}\)(PO\(_4\))\(_6\)(OH\(_2\)) nanoparticles, the nano-sized Ca\(_{10}\)(PO\(_4\))\(_6\)(OH\(_2\)) particles had negative or diamagnetic susceptibility value which is in line with research conducted by Chandra et al. [25].

The magnetic property changes of the nano-sized Ca\(_{10}\)(PO\(_4\))\(_6\)(OH\(_2\)) particles are strictly related to the successful dopant substituted to Ca\(^{2+}\). The definite reason for Gd\(^{3+}\) ion successfully distributed is because the Gd\(^{3+}\) ion had the same charge type as and almost similar size to Ca\(^{2+}\) ion. According to Langevin theory, the magnetic susceptibility value of the mass is proportional to the magnetic moment of material. The increasing doping composition improved the susceptibility of the material. One of the factors causing the increase in string value of magnetic field was magnetization of material determined by finding the susceptibility value in the volume. The magnetic susceptibility value was proportional to the magnetic moment of the material as shown by Langevin equation. Therefore, theoretically, the higher the doping value, the susceptibility will enhance as well. This case was also indicated by experimental measuring showing the linearity of correlation between susceptibility and a lot of doping.
Biocompatibility was observed by determining the toxicity of the nano-sized Ca_{10}(PO_{4})_{6}(OH)_{2} particles by recording the intestinal contraction of the experimental animal. The contraction was recorded at the 5th – 15th minutes and it indicated that all prepared samples in this work were biocompatible. The recording was conducted since at those periods organ undergoes constant contraction with maximum amplitude [26]. Specifically, from the recording results, the intestine contracted proven by wave peaks. In general, the record of intestinal contraction is standard in the same time range; many intestinal contractions doped do not change significantly. This case means that organ does not refuse due to the doping composition provided. Meanwhile, the similar research results of Ashokan et al. showed that nano-sized Ca_{10}(PO_{4})_{6}(OH)_{2} particles were biocompatible in the doping
of 4.4% Gd<sup>3+</sup> ion [27]. Visually, the voltage and noise decreased in intestinal contraction after being given the nano-sized Ca<sub>x</sub>(PO<sub>4</sub>)<sub>6</sub>(OH) particles. However, by the addition of x value, the voltage resulted enhanced. The change of this voltage was closely related to energy and intensity resulted, in this case was correlated to the strength of contraction. Meanwhile, the noise in the addition of the nano-sized Ca<sub>x</sub>(PO<sub>4</sub>)<sub>6</sub>(OH) particles indicated that body responded other things entering into body. The process of drug delivery into body is also called drug bioavailability (stating the speed and the number of active drugs reaching the site) [28]. This process is explained through theory of receptor occupancy where the intensity of drug effect is proportional to receptor occupied and will be maximum if all receptors are occupied by drug. In this research, the higher the number of dopants provided, the organ contraction response increased but it was still normal. This case shows the curve of the nano-sized Ca<sub>x</sub>(PO<sub>4</sub>)<sub>6</sub>(OH) particles is proportional to the comparator curve (contraction in normal intestine) so that the product compared is considered biocompatible to the body. A physical review can be seen through the graph between time and voltage. The research results indicated that the resulted voltage ranged from 0.17 – 0.23 volt. Therefore, the nano-sized Ca<sub>x</sub>(PO<sub>4</sub>)<sub>6</sub>(OH) particles were accepted by body since the resulted voltage was about 0.2 volt, not far from the contraction in standard intestine. The higher voltage value showed the body rejection of drug. Hence, it can be concluded that the prepared nano-sized Ca<sub>x</sub>(PO<sub>4</sub>)<sub>6</sub>(OH) particles in this work exhibited biocompatible.

4. Conclusion

The nano-sized Ca<sub>x</sub>(PO<sub>4</sub>)<sub>6</sub>(OH) particles (0% ≤ x ≤ 1.75%) were successfully synthesized by using a hydrolysis method based on natural resource. XRD analysis resulted in information that the higher the composition of Gd<sup>3+</sup> ion, the decrease the lattice parameters and crystal volume of the nano-sized Ca<sub>x</sub>(PO<sub>4</sub>)<sub>6</sub>(OH) particles. In addition, the susceptibility of the nano-sized Ca<sub>x</sub>(PO<sub>4</sub>)<sub>6</sub>(OH) particles improved as the addition of the composition of Gd<sup>3+</sup> ion. The magnetic properties of the nano-sized Ca<sub>x</sub>(PO<sub>4</sub>)<sub>6</sub>(OH) particles changed from diamagnetic to paramagnetic after being inserted by Gd<sup>3+</sup> ion. Moreover, the nano-sized Ca<sub>x</sub>(PO<sub>4</sub>)<sub>6</sub>(OH) particles were biocompatible and opened new potency for biomedical application.

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