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Polyol synthesis of gadolinium nanoparticles: A preliminary study

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Abstract

Gadolinium nanoparticles (Gd NPs) are considered an alternative strategy to utilize gadolinium as a Magnetic Resonance Imaging (MRI) contrast agent. In addition, this approach offers several advantages, such as enhancing the T1-relaxivity by employing a small amount of gadolinium and conveniently modifying with several molecules (e.g., folic acid as a targeting agent). This preliminary preparation study aims to observe the different synthesis temperatures of gadolinium nanoparticles *via* the polyol method (e.g., at 180, 185, and 190ºC). Diethylene glycol (DEG) molecule was employed as a solvent to help the formation of nanoparticles. The prepared Gd NPs were then purified and further characterized with several instruments, such as a Particle Size Analyzer (PSA), Scanning Electron Microscope (SEM), Thermal Emission Morphology (TEM), Dynamic Laser Scattering (DLS), Thermal Gravimetric Analyzer (TGA), and Zeta Potential instruments. The results show that 185ºC was the optimum temperature to be applied for the synthesis Gd NPs *via* the polyol method based on PSA analysis. In addition, the spherical Gd NPs with a size below 100 nm were revealed from SEM and TEM characterization. Furthermore, the zeta potential number of Gd NPs also exhibited the good stability of Gd NPs. However, the impurities peak from the organic materials such as unreacted DEG still appeared in SEM or TGA results. Therefore, further purification will be performed in order to utilize these Gd NPs for biomedical applications.

Keywords: Gadolinium, Nanoparticles, Polyol method, Magnetic Resonance Imaging; A Preliminary Study.

Full length article **Corresponding Author*, e-mail: retna.fauzia@unpad.ac.id

1. Introduction

Cancer is the second most frequent cause of death with around 10 million lethal cases happening yearly in worldwide (WHO Data). In order to reduce these high mortality numbers, early cancer diagnosis will help to find the more accurate treatment [1]. There are several cancer imaging modalities techniques, such as Magnetic Resonance Imaging (MRI), Computed Tomography (CT), Ultrasonography (US), Positron and Single Photon Emission Tomographies (PET and SPECT, respectively). MRI is the most powerful non-invasive diagnosis technique which offers the high resolution of soft tissue images. In addition, compared to PET and SPECT, there will be no ionizing radiation involved on MRI process, therefore it will not cause any harm to the patient [2]. In addition, in order to enhance the MRI imaging results, the utilization of MRI contrast agents is a must [3]. MRI contrast agents based on paramagnetic gadolinium complex (e.g. gadolinium diethylenetriamine-pentaacetate or Gd(III)-DTPA) has been already available commercially [4]. However, its toxicity currently still on debate due to its Gd^{3+}dissociation from Gd(III)-DTPA complex [5,6]. Therefore, gadolinium nanoparticles (Gd NPs) can be considered as an alternative strategy to overcome this limitation. Gd NPs offer the better stability compared Gd-complexes MRI contrast agent, hence result in lower Gd^{3+} toxicity as well as higher T1-imaging results [7]. Gd NPs with diameter below 100 nm also have an enhanced permeability and retention (EPR) effect which will lead to high accumulation on the cancer cells [8,9]. Gadolinium as nanoparticles is also conveniently modified with several molecules, such as polyethyleneglycol (PEG) to enhance their biocompatibility[10] or active targeting agents such as folic acid [11] and antibody [12] to enable receptor binding on specific cancer of interest. There are several methods to synthesis nanoparticles, such as polyol [13,14], sol-gel [15], co-precipitation [16], hydrothermal [10,17], solvothermal [18], and thermal decomposition [19] Among these, polyol synthesis method offers the fast and convenient synthesis procedure without an extreme temperature involved [20,21]. Their diameter size can also be adjusted with the amount temperatures achieved for the final reaction of metals and poly-alcohol used. In this preliminary study, Gd NPs were

prepared using polyol method with three different synthesis temperatures in order to obtain their optimum condition. Diethylene glycol (DEG) as a poly-alcohol was employed as a solvent to facilitate the formation of Gd NPs. Gd NPs were then characterized with several instruments, such as a Particle Size Analyzer (PSA) and Scanning Electron Microscope (SEM) for their size and morphology investigation, Dynamic Light Scattering (DLS), Thermal Gravimetric Analyzer (TGA) and Zeta Potential instruments were also employed to observe their surface functionalization and impurities. These several analyses were performed in order to utilize these Gd NPs for further biomedical applications.

2. Materials and methods

2.1. Materials

Gadolinium chloride hexahydrate ($GdCl_3.6H_2O$), sodium hydroxide (NaOH), and diethyleneglycol (DEG) were purchased from Sigma Aldrich. Pure H_2O was acquired from a Mili-Q system.

2.2. Methods

2.2.1. Preparation of gadolinium and sodium hydroxide solution

2 mmol of $GdCl_3.6H_2O$ and 2.5 mmol of NaOH were dissolved using sonicator bath in 7.5 mL and 2.5 mL of DEG for 2h, respectively. The prepared solutions were then stored at room temperature.

2.2.2. Preparation of Gd NPs via polyol method

Firstly, the prepared gadolinium and sodium hydroxide solution were transferred into the three different reflux flask and labeled as Gd NPs 180, Gd NPs 185, and Gd NPs 190. Each solution was heated at 60°C for 30 min to form a clear mixing solution. The mixing solution was slowly heated to 140°C for 1h and then to 180 (Gd NPs 180), 185 (Gd NPs 185), and 190°C (Gd NPs 190) for 4h. The Gd NPs solution was cooled at room temperature and then analysed using Particle Size Analyzer (PSA, Beckman Counter LS 13 320) to determine the optimum condition of Gd NPs syntesis .

2.2.3. Purification of Gd NPs

The Gd NPs solution was then centrifuged at 1750 RPM for 30 min. The resulted filtrate was then dialysed with water for 24h and then futher filtered with membrane Polyethersulfonate (PES) 0.22 um for Gd NPs at the optimum reflux condition. The pure Gd NPs solution was freeze dried for further characterization.

2.2.4. Characterization of Gd NPs

The Gd NPs were characterized with several instruments, such as Scanning Electron Microscope (SEM, Hitachi TM3000) and Transmission Electron Microscope (TEM, JEOL JEM-2100) to investigate the size and morphology of Gd NPs, Dinamic Light Scattering-Zeta Potential (Malvern Zetasizer NanoZS) to observe the stability and the presence of Gd NPs impurities.

3. Results and Discussions

3.1. Synthesis of Gd NPs via polyol method

Gd NPs were synthesized via polyol due to its convenient synthesis route for preparation nanoparticles. Polyol method employs a conventional synthesis system without using extremely high temperature or any complicated instrument for its synthesis [21]. Furthermore, the successful synthesis *via* polyol route is mainly determined by several factors, such as the solvent used, the sample's concentration, and the optimum reflux temperature applied. In this polyol synthesis, diethylene glycol (DEG) was utilized not only as a solvent but also as a reducing agent [14] . Its high boiling point will also not be affected in the time of their formation process. Hence, it will help to prevent the agglomeration of Gd NPs during synthesis. The employment of DEG as a polyol molecule will also determine the nucleation and growth process of nanoparticles. Its oxidation will also result on aldehyde and ketone molecules which responsible to the reduction process of metal nanoparticles [14].Click or tap here to enter text. The $GdCl_3.6H_2O$ as a gadolinium source in DEG solvent was then put into a round bottom flask that had been filled with a magnetic stir bar. The reflux process was controlled for 30 minutes and changes in temperature are recorded every 15 minutes. The aim of using a temperature of 60°C was to ensure that $GdCl_3.6H_2O$ in DEG solvent had been completely dissolved. The reflux process was continued by increasing the temperature to 140°C gradually and slowly. The NaOH solution was then added using a syringe into a round bottom flask. The reflux process was continued for 1 hour and the thermometer temperature was ensured to be constant at 140°C. The temperature of 140°C was chosen because this temperature is the optimum temperature for dissolving the $GdCl_3.6H_2O$ and NaOH solutions. The color of the solution resulting from adding NaOH is clear and transparent. This indicates that NaOH had been completely dissolved in the synthesis of Gd NPs. At this elevated temperature increase, the nucleation step occurs. The nucleation began with the reactants being dissolved in a solvent, followed by the emergence of atoms due to chemical reactions between the reactants, so that the atomic concentration increases to the saturation level. When it reaches the saturation level, the atoms aggregate into a smaller form (nucleus) through a nucleation process which takes place quickly and causes the atomic concentration to drop below the saturation level, where in this condition, no additional nucleation process occurs [20]. After the reflux process lasts for 1 hour at a temperature of 140°C, the temperature was increased by varying the temperature at 180, 185, and 190°C with a reflux time of 4 hours. At this stage, nanoparticle growth occurs. Nuclei that have reached a point below the saturation level develop into nanoparticles and their size increases until atomic equilibrium occurs between the nanoparticle surface and the solution. The reaction for the synthesis of Gd NPs was shown in the following reaction [13]:

$$GdCl_3.6H_2O + 3NaOH \rightarrow Gd(OH)_3 + 3NaCl \quad (1)$$

$$2Gd(OH)_3 \rightarrow Gd_2O_3 + 3H_2O \quad (2)$$

Based on reaction at Eq.(1), there has been a substitution reaction with NaOH between the hydroxyl group and chloride ion, resulting in the formation of gadolinium hydroxide ($Gd(OH)_3$) and the side product in the form of NaCl. In the formation of $Gd(OH)_3$, a dehydration reaction has occurred. The dehydration reaction is a type of elimination reaction, namely the reduction of a water molecule from an alcohol molecule (containing an –OH group).

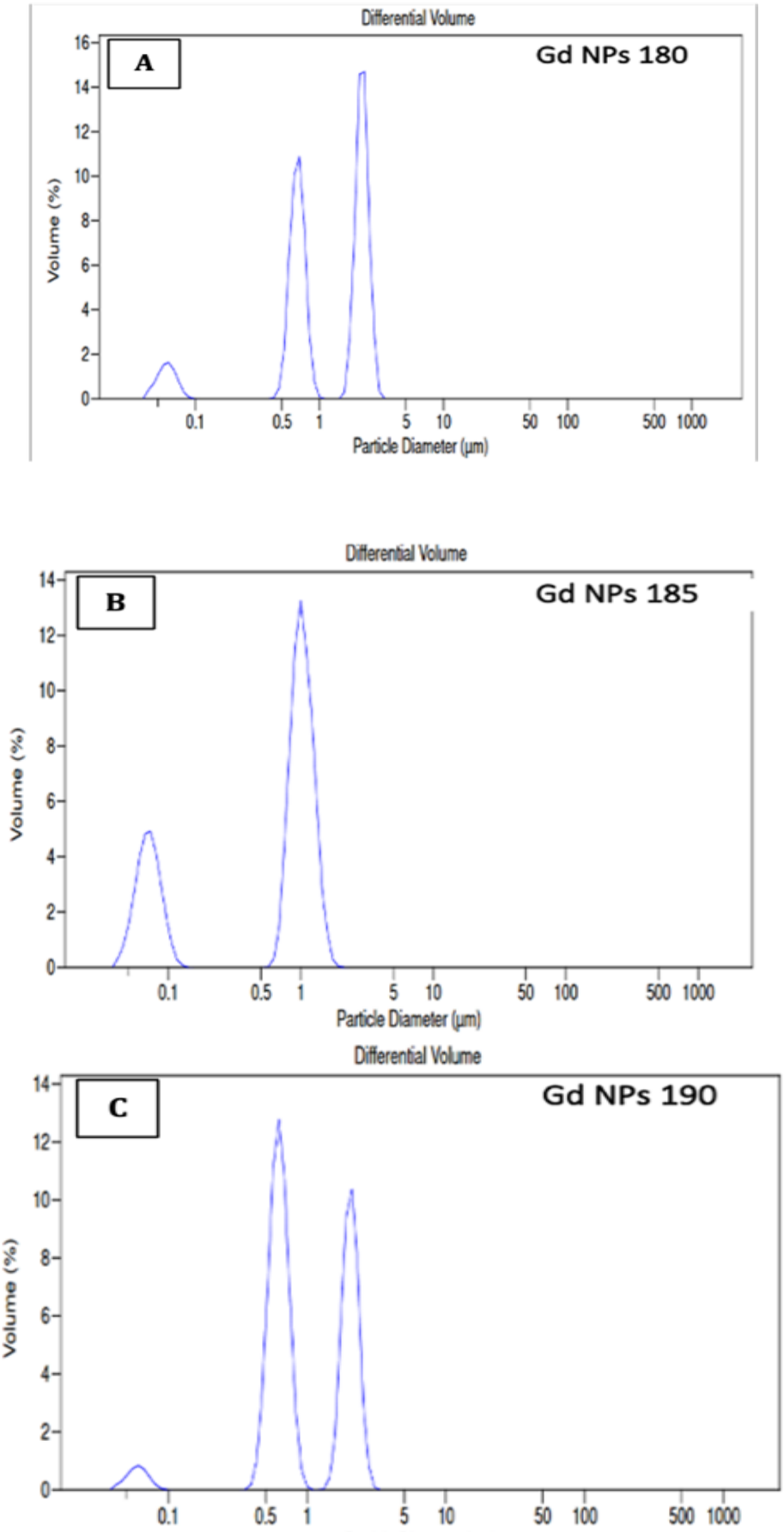

Figure 1: PSA results of a preliminary study of Gd NPs polyol synthesis. Gd NPs synthesis by using temperature of 180ºC – Gd NPs 180 (A), 185ºC – Gd NPs 185 (B), and 190ºC – Gd NPs 190 (C)

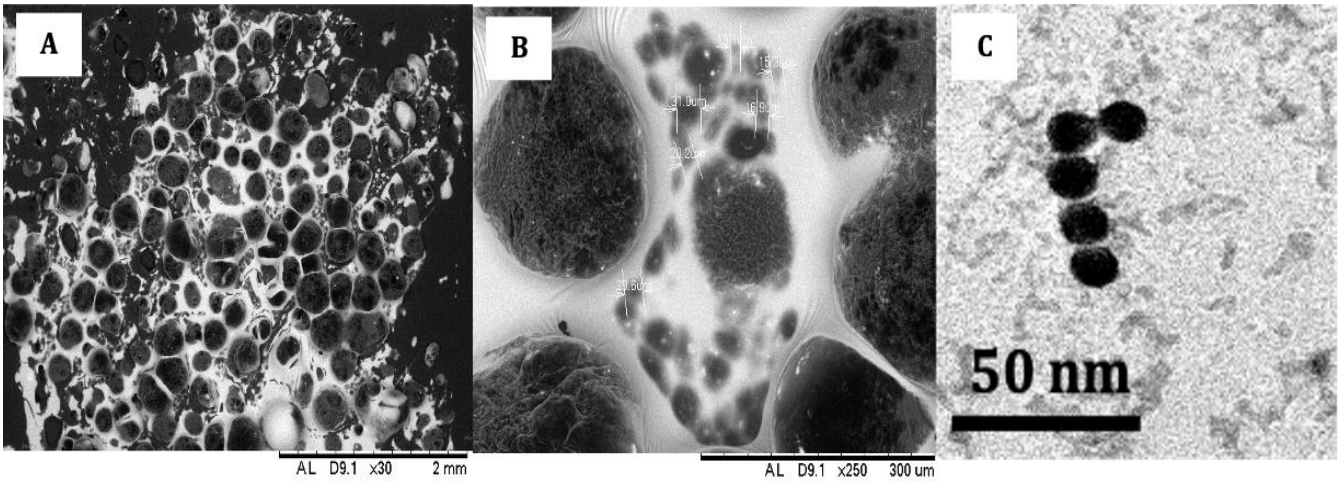

Figure 2: Gd NPs 185 SEM and TEM images. SEM 30X (A), SEM 250X magnification (B) and TEM (C).

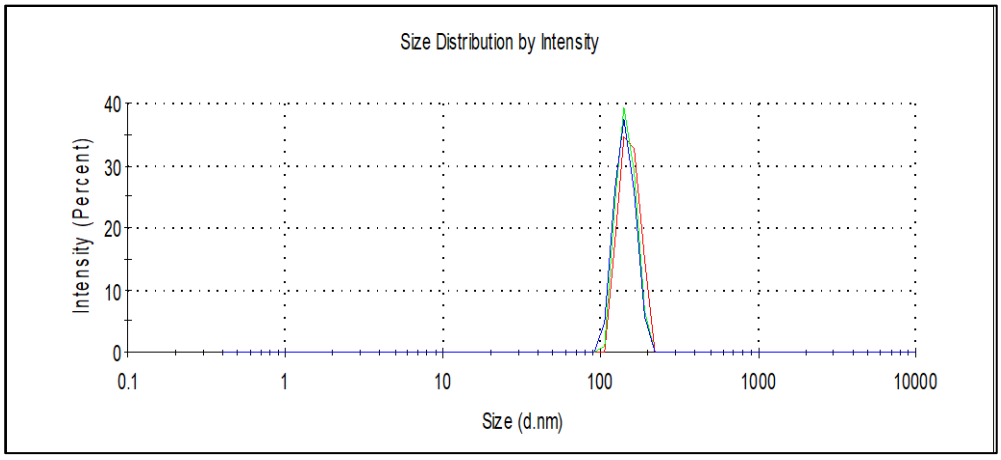

Figure 3: DLS size diameter distribution of Gd NPs 185.

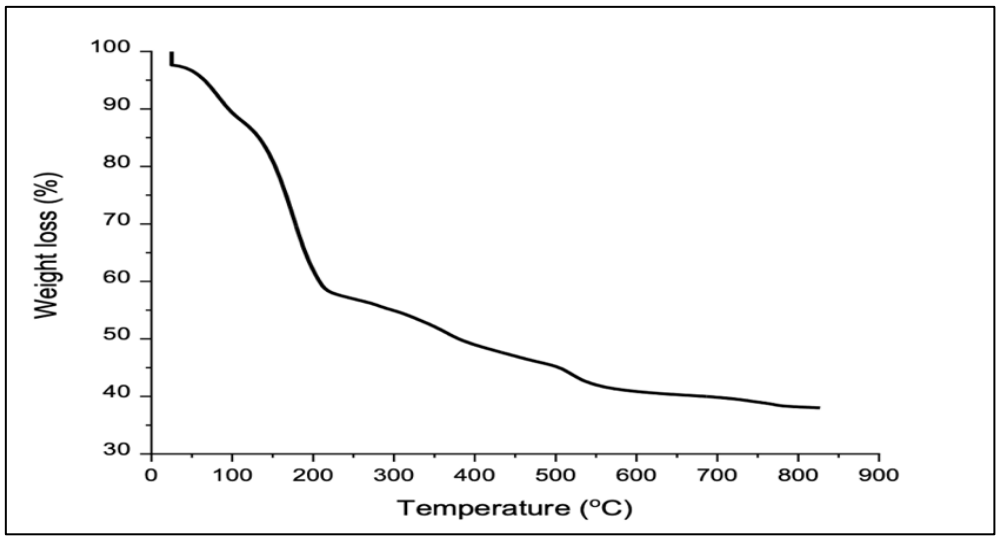

Figure 4: TGA curves of Gd NPs 185.

Table 1: The yield data of Gd NPs mass for each polyol reflux temperature.

Reflux temperature (°C)	Mass of Gd NPs (g)
180	84
185	165
190	71

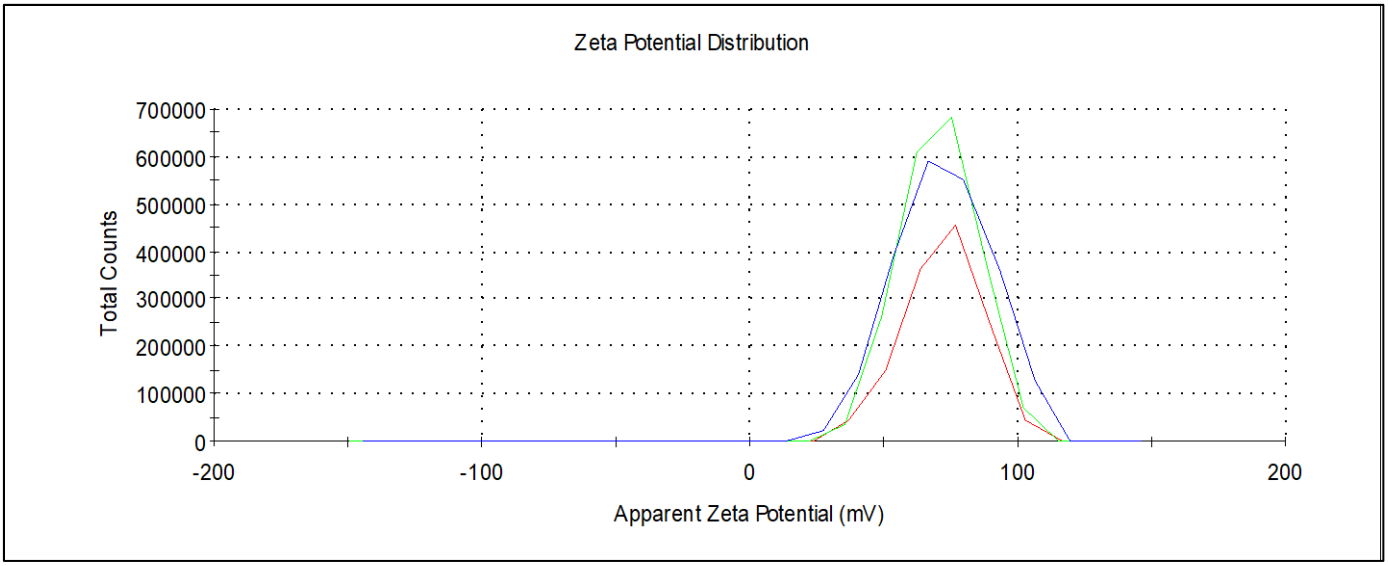

Figure 5: Zeta potential results of Gd NPs 185.

Meanwhile, the formation of a by-product in the form of NaCl or DEG were characterized by the formation of a white precipitate during the synthesis process at high temperatures. By utilizing high temperatures and periodic increases in the reflux process, $Gd(OH)_3$ undergoes a dehydration reaction to produce products in the form of Gd_2O_3 and H_2O according to reaction at Eq.(2).

3.2. Purification of Gd NPs

The first purification step of Gd NPs began with a centrifugation process at a speed of 1750 rpm for 30 minutes. Centrifugation is a process of separating insoluble mixtures by utilizing centrifugal force which is based on the weight of the particles relative to their density. After the Gd NPs were centrifuged, no sediment was found in the centrifuge tube. This indicates that the Gd NPs synthesis process that has been carried out gives good results because no precipitate is formed in the solution. The next purification process is purifying Gd NPs via dialysis. Dialysis must be performed to purify Gd NPs from their impurities, for instances the unreacted materials during the synthesis. A membrane with a molecular weight limit of 1000 Dalton (1000 MWCO) was utilized for their nanofiltration process. DEG which has a molecular weight of less than 1000 Daltons is expected to come out of the membrane, hence the Gd NPs which have a molecular weight of more than 1000 Daltons are expected to remain in the membrane. Afterwards, Gd NPs were freeze dried in order to obtain the solid form of Gd NPs and then calculated for their yield of nanoparticles (Table 1).

3.3. Characterization of Gd NPs

3.3.1. PSA results

Particle Size Analysis (PSA) aims to determine the size distribution of nanoparticles in a preliminary result of Gd NPs polyol synthesis. PSA has been carried out with temperature variations (180, 185 and 190oC) as shown in Figure 1. Figure 1A-C shows the results of the PSA analysis for different polyol reflux temperature of Gd NPs formation (180, 185, and 190°C). which states that Gd NPs have been distributed into nano sizes, but they are still in small amounts. This is due to the influence of temperature on the reflux process which results in imperfect nanoparticles formation. The largest size distribution of Gd NPs was found at a temperature variation of 185°C with a volume percentage of 30%. We can conclude that 185°C was the optimum condition for Gd NPs polyol synthesis. These Gd NPs (Gd NPs 185) were then further filtered using membrane PES 0.22 um to obtain the only Gd NPs at nano sizes distribution.

3.3.2. SEM, TEM, DLS and TGA analysis

The Gd NPs 185 samples were then further analyzed using a SEM and TEM instrument to determine the surface morphology and size of the nanoparticles. It can be seen that the morphology of Gd NPs 185 had spherical shape with smallest sizes of 94 and 20 nm based on SEM and TEM analysis, respectively (Figure 2A-C). Morphological shape is very crucial on nanoparticles synthesis, because it will affect the further cells internalization for their biomedical applications. In addition, DLS analysis was also conducted to observe the size diameter of Gd NPs (Figure 3). Apparently,

the size diameter above 100 nm was discovered supposed from unreacted DEG on Gd NPs which was also observed on white artifacts colors from SEM and TEM images. Assumed due to the too immediate dialysis time process which must be prolong for further purification step. Furthermore, TGA measurement was also performed in order to observe the presence of impurities on Gd NPs. It was also observed the mass loss of 15% organic materials at 200-500ºC which presumed as the unreacted of DEG molecules during the polyol synthesis (Figure 4).

3.3.3. Zeta potential measurement

In order to further observe the stability of colloidal dispersion of Gd NPs, zeta potential analysis was also performed. From the Figure 5, it was calculated that the zeta potential of Gd NPs 185 was above +70 mV. It was concluded that Gd NPs 185 have an excellent stability as water dispersion nanoparticles. This property is very important for further utilization of Gd NPs in biomedical applications.

4. Conclusions

The optimum condition of Gd NPs polyol synthesis was successfully synthesized at temperature of 185ºC based on PSA control analysis with approximately yield of 165 mg Gd NPs. The SEM and TEM observation results show that Gd NPs have a spherical shape with the smallest size at 94 and 20 nm, respectively. However, DEG as an unreacted material is still observed due to the short dialysis process. Therefore, the further purification procedure must be applied for Gd NPs polyol synthesis. In spite of them, based on zeta potential results, Gd NPs have already showed good stability which is also crucial for their further biomedical applications.

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