Synthesis and Characterization of Fe₃O₄ Nanoparticles using Polyvinyl Alcohol (PVA) as Capping Agent and Glutaraldehyde (GA) as Crosslinker

Lale Budi Hutami Rahayu¹, Ika Oktavia Wulandari¹, Djoko Herry Santjojo², Akhmad Sabarudin¹*
¹Department of Chemistry, Faculty of Science, Brawijaya University, Jl Veteran 65145 Malang, Indonesia
²Department of Physics, Faculty of Science, Brawijaya University, Jl Veteran 65145 Malang, Indonesia
Email: sabarjpn@ub.ac.id

Abstract. The use of polyvinyl alcohol (PVA) as a capping agent and glutaraldehyde (GA) as a crosslinker for a synthesis of magnetite (Fe₃O₄) nanoparticles is able to reduce agglomeration of produced Fe₃O₄. Additionally, oxidation of Fe₃O₄ by air could be avoided. The synthesis is carried out in two steps: first step, magnetite (Fe₃O₄) nanoparticles were prepared by dissolving the FeCl₃.6H₂O and FeCl₂.4H₂O in alkaline media (NH₃·H₂O). The second step, magnetite nanoparticles were coated with polyvinyl alcohol (PVA) and glutaraldehyde (GA) to obtain Fe₃O₄-PVA-GA. The latter material was then characterized by FTIR to determine the typical functional groups of magnetite coated with PVA-GA. X-ray Diffraction analysis was used to determine structure and size of crystal as well as the percentage of magnetite produced. It was found that the produced nanoparticles have crystal sizes around 4-9 nm with the cubic crystal structure. The percentage of magnetite phase increases when the amount of glutaraldehyde increased. SEM-EDX was employed to assess the surface morphology and elemental composition of the resulted nanoparticles. The magnetic character of the magnetite and Fe₃O₄-PVA-GA were studied using Electron Spin Resonance.

1. Introduction
Nanoparticles are nano-sized materials that can be applied in several fields such as industrial and biomedical fields. Applications of nanoparticles in the biomedical field to enhance magnetic resonance imaging (MRI) magnification image and as drug delivery system for cancer therapy [1]. The use of the drug delivery system is capable of delivering the drug to the target cell [2]. The presence of magnetic properties in magnetic materials can be used to transmit targeted drugs using an external magnetic field. Iron oxide is a magnetic material that has low toxicity, cheap, biocompatible, biodegradable and unique magnetic properties [3], [4]. The magnetic field in the iron oxide occurs because of the four unpaired electrons in the d orbitals [5]. Iron oxide can be utilized as a target drug delivery system if it...
has a size below 20 nm. Some researchers have reported their works on iron oxide nanoparticles by varying the factors that affect the properties of nanoparticles [2], [6], [7].

The synthesis of magnetite nanoparticles is largely chemical made to obtain nanoparticles in small size more easily. The synthesis method with co-precipitation at room temperature is known to be the least expensive, easy method and obtains a high percentage of magnetite (Fe₃O₄) [8]. Magnetite nanoparticles have a low stability that tends to agglomerate and easy to oxidate. The agglomeration properties of magnetite nanoparticles influenced the presence of Van der Waals forces and the pull of magnetic dipole [9], [10]. To prevent aggregation of magnetite nanoparticles, a surface modification or a capping agent capable of conjugating with therapeutic (drug) molecules [1] is required. Usually, nanoparticle coatings may use polymers or surfactants, since the presence of crosslinked polymers can prevent coagulation and produce monodisperse particles [11].

Polyvinyl alcohol (PVA) can be used to stabilize nanoparticles due to their biodegradable and hydrophilic properties [12]. However, the hydrophilic nature of PVA can reduce its effectiveness as a capping agent because drug release can occur before it reaches the target cell associated with its interaction with the fluid in the body. Thus a crosslinker is required to inhibit the degradation of the nanoparticle-polymer so that degradation can occur after the drug reaches the target cell. Glutaraldehyde (GA) can be used as a crosslinker because it is stable in acidic conditions so that it can be obtained a nanoparticle that has a high stability in acidic conditions, and can be degraded in body fluids after reaching the target cell. The synthesis of nanoparticles using PVA as a capping agent and glutaraldehyde as a crosslinker has been done by Kim [13] and developed by Wahid [14]. The presence of PVA and glutaraldehyde will produce better Fe₃O₄ nanoparticles.

2. Materials and Methods

2.1. Chemicals and Instrumentation

The material used was Iron (III) chloride hexahydrate, Iron (II) chloride tetrahydrate, glutaraldehyde (Sigma Aldrich), from polyvinyl alcohol (PVA, Mw 3500) technical grade (Merck Indonesia), and aqueous ammonium hydroxide.

FTIR (Fourier Transform Infrared) spectrophotometer (Shimadzu FTIR QP89500, KBr pellet in wavenumber range 4000-400 cm⁻¹). The FTIR analysis used to determine the functional groups of nanoparticle samples. Freeze Dry used to remove water molecules on nanoparticle samples (35 XL Virtis SP Scientific) at temperature condition under -40°C and pressure up to ≤ 20 mT. ESR (electron spin resonance) used to determine the magnetic character of the nanoparticle materials (Leybold Didactic GMBH). SEM-EDX (Scanning electron microscopy) analysis used to determination of morphology and elemental composition in nanoparticles (Hitachi SU 3500 with magnification around 1000 - 20.000 kX). XRD analysis used to measure the crystal size and lattice parameters of Fe₃O₄ nanoparticles and the existence of Fe₂O₃ from nanoparticle samples were carried out using Diffractometer system XPERT-PRO with Cu-Kα λ= 1.5406 Å radiation was used for X-ray powder diffraction measurements. XRD data was recorded across a 2θ range of 5° to 60° using a step size of 0.001°.

2.2. Synthesis Procedure

The synthesis of Fe₃O₄ nanoparticles by co-precipitation method follows a procedure performed by Kim et al., (2012) with a briefly described modification wherein 0.125 g of FeCl₃·6H₂O and 0.35 g FeCl₂·6H₂O with molar ratio (1:2) dissolved by 100 mL aquadem in a beaker glass and stirred at room temperature at 180 rpm under vacuum, added 5 mL of 3M NH₄OH solution dropwise by syringe pump at a rate of 10 mL/h while stirring using a magnetic stirrer, resulting in a Fe₃O₄ precipitate. Further 5 mL of PVA solution with concentration (5 g of PVA dissolved in 70 mL aquadem) dropwise at 10 mL/h and glutaraldehyde solution (50, and 150 μL) while stirring. The resulting precipitate is then separated by pulling it using a bar magnet placed beneath the beaker glass. The precipitate is then washed using aquadem until neutral pH or ± 7. The sample is then dried in a freeze dryer and the
sample is stored in the desiccator before further analysis. The resulting samples are labeled with the name \( \text{Fe}_3\text{O}_4\)-PVA\(_5\)-GA\(_{50}\) and \( \text{Fe}_3\text{O}_4\)-PVA\(_5\)-GA\(_{150}\). \( \text{Fe}_3\text{O}_4 \) nanoparticles were produced using the same method without the addition of PVA and glutaraldehyde.

3. Results and Discussions
The aim of this study was to synthesize \( \text{Fe}_3\text{O}_4 \) nanoparticles using Iron (II) chloride tetrahydrate hexahydrate and Iron (III) chloride hexahydrate with ratio mole (1:2) in alkali medium. The nanoparticles were coated with PVA to avoid oxidizing easily, and glutaraldehyde to stabilize the nanoparticles. To determine the effect of added glutaraldehyde, mole variation of glutaraldehyde were added to the sample of the nanoparticles were added. Interactions occurring on the surface of nanoparticles in the presence of PVA and glutaraldehyde can be seen in scheme 1. The presence of hydroxyl groups on the \( \text{Fe}_3\text{O}_4 \) surface serves to stabilize the surface of \( \text{Fe}_3\text{O}_4 \). Then, the presence of glutaraldehyde will bind to one PVA hydroxyl group with another PVA hydroxyl group forming a crosslinker. Glutaraldehyde may act as a crosslinker because in glutaraldehyde there are two carbonyl groups located at the ends of the glutaraldehyde group. In addition, the presence of glutaraldehyde will also produce an active compound glutaraldehyde which will directly bind to the surface of the nanoparticles, so it is important to organize the amount of glutaraldehyde mole [15].

![Scheme 1. Interaction of crosslinker formation between \( \text{Fe}_3\text{O}_4 \) nanoparticles with presence of PVA and glutaraldehyde]

All synthesized nanoparticle samples indicate the presence of magnetic properties in aqueous water when the sample is closer to the external magnetic field as can be seen in Figure 1. After the sample is dried, the bare \( \text{Fe}_3\text{O}_4 \) and coated with PVA and GA shows different colors. The \( \text{Fe}_3\text{O}_4 \) nanoparticles are black in shape as sand, while the coated nanoparticles are blackish brown with finer shapes, as can be seen in Figure 2.
Figure 1. Fe$_3$O$_4$ nanoparticles (a) before and (b) exposed closer to an external magnetic field

Figure 2. The forms of Fe$_3$O$_4$ nanoparticles (a) bare (a) after coated PVA and glutaraldehyde

SEM analysis shows that the surface morphology of bare Fe$_3$O$_4$ and coated are shaped cluster (see Figure 3). This is due to the very small size of the crystals of Fe$_3$O$_4$ which is below 20 nm, so the surface energy is still very high. From Figure 3 it is known that the presence of Fe$_3$O$_4$ can be seen in the presence of small particles of light/white, due to the presence of high Fe atomic number.

Figure 3. SEM micrographs for Fe$_3$O$_4$ (a) bare in 10.000x magnification (b) bare in 20.000x magnification (c) after coated in 10.000x magnification and (d) after coated in 20.000x magnification

EDX analysis found that elements contained in the sample are Fe, O, and C. The presence of C in Fe$_3$O$_4$ bare is due to the sample being analyze coated with carbon. The EDX spectral peaks are in the region binding energy of Fe in the range of 0.8, 6.3, and 6.8 keV [16], with the percentage of atomic mass for bare Fe$_3$O$_4$, Fe$_3$O$_4$-PVA$_5$-GA$_{50}$ and Fe$_3$O$_4$-PVA$_5$-GA$_{150}$ presented in Table 1.
Table 1. The percentage of atomic mass for nanoparticles samples with the amount of mole of glutaraldehyde added

| Samples                  | %Fe   | %O    | %C    |
|--------------------------|-------|-------|-------|
| Bare Fe₃O₄               | 61,45 | 32    | 6,55  |
| Fe₃O₄-PVA₅-GA₅₀         | 34,73 | 46,20 | 19,06 |
| Fe₃O₄-PVA₅-GA₁₅₀        | 41,00 | 48,09 | 10,91 |

Diffractionogram for nanoparticle samples of XRD characterization can be seen in Figure 4 and the crystal size was determined by Williamson-Hall calculations [17]. Figure 4 exhibits all samples where the diffraction peaks in the hkl plane are (220), (311), (222), (400), (422), (333) respectively corresponding to the COD standard (Crystallography Open Database) number # 96-900-2320 for Fe₃O₄. The Fe₃O₄ crystal is composed of Fe³⁺ composed of the tetrahedral side and half of Fe²⁺ from the caved octahedral side. The crystal size for bare Fe₃O₄, Fe₃O₄-PVA₅-GA₅₀, and Fe₃O₄-PVA₅-GA₁₅₀ were respectively 4.66; 5.96; and 7.85 nm. So it can be seen that nanoparticles that are coated PVA and glutaraldehyde will increase the crystal size of Fe₃O₄.

![Diffractionogram for bare Fe₃O₄, Fe₃O₄-PVA₅-GA₅₀ and Fe₃O₄-PVA₅-GA₁₅₀ nanoparticles](image)

Figure 4. Diffracrogram for bare Fe₃O₄, Fe₃O₄-PVA₅-GA₅₀ and Fe₃O₄-PVA₅-GA₁₅₀ nanoparticles

The percentage obtained in all samples, the highest percentage of magnetite, followed by the percentage of maghemite and hematite. The amount of mole of glutaraldehyde added, the percentage of magnetite increases, which can be seen in Figure 5.
Figure 5. The percentage of magnetite from bare Fe$_3$O$_4$, Fe$_3$O$_4$-PVA$_5$-GA$_{50}$, and Fe$_3$O$_4$-PVA$_5$-GA$_{150}$ nanoparticles.

Analysis of magnetic characters from nanoparticle samples by calculating the g-lande factor values obtained from ESR measurements. The g-lande factor values for bare Fe$_3$O$_4$, Fe$_3$O$_4$-PVA$_5$-GA$_{50}$ and Fe$_3$O$_4$-PVA$_5$-GA$_{150}$ nanoparticles were 1.94541; 1.90611; and 1.91903. From the g-lande value obtained, it is known that this value is close to the g factor value for Fe tend to ferromagnetic.

Figure 6 shows FTIR spectra from samples of Fe$_3$O$_4$ bare nanoparticles as well as coated PVA and glutaraldehyde. The fingerprint region for Fe-O appears in sharp areas of 450 cm$^{-1}$ and ~ 585 cm$^{-1}$ indicating the presence of magnetite compounds [18] and in the sample there is also a slight peak in the absorbent band of ~ 623 cm$^{-1}$, indicating that maghemite is also formed [19]. The presence of maghemite compounds that are also detected due to Fe$_3$O$_4$ that is not coated will easily oxidize in the presence of oxygen. It is known that in the presence of PVA and glutaraldehyde, the absorption bands in the area of ~ 623 cm$^{-1}$ are decreasing as the amount of mole of glutaraldehyde increased.

Figure 6. The Spectra of FTIR of bare Fe$_3$O$_4$ and coated with PVA and glutaraldehyde.
4. Conclusion
This study described the effect of the added number of glutaraldehyde moles to Fe₃O₄ nanoparticles in the presence of PVA as a capping agent. In this study, the crystal size ranges from 4 to 9 nm with cubic crystal structure with the percentage of magnetite obtained on the Fe₃O₄-PVA₃-GA₁₅₀ sample of 89.20% which is the maximum percentage of the three samples. So it can be concluded that the addition of glutaraldehyde in Fe₃O₄ will increase the percentage of magnetite obtained.

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