A synthesis of polyethylene glycol (PEG)-coated magnetite Fe₃O₄ nanoparticles and their characteristics for enhancement of biosensor

Ganesha Antarnusa¹ and Edi Suharyadi²

¹ Department of Physics Education, Universitas Sultan Ageng Tirtayasa, Indonesia
² Department of Physics, Universitas Gadjah Mada, Yogyakarta, Indonesia
E-mail: ganesha.antarnusa@untirta.ac.id

Keywords: polyethylene glycol (PEG), co-precipitation method, biosensor applications, magnetite Fe₃O₄, nanoparticles

Abstract
The magnetite Fe₃O₄ nanoparticles were synthesized by using chemical co-precipitation method and these nanoparticles were successfully coated by polyethylene glycol (PEG) with variation concentrations of PEG. The magnetite Fe₃O₄ nanoparticles used as a bimolecular label (nano-tags), exhibiting a soft magnetic behavior with magnetization (Mₛ) of 77.16 emu g⁻¹ and coercivity (Hᵥ) of 50 Oe respectively. The polyethylene glycol (PEG) was used as a biocompatible polymer. The x-ray diffraction (XRD) patterns of the Fe₃O₄ showed that Fe₃O₄ was well crystallized. It also confirmed the existence of inverse spinel. The diffraction peak of 35.4° was used to calculate the crystallite size. The estimation of Fe₃O₄ average crystallite size is 12 nm, while the PEG-coated Fe₃O₄ nanoparticles is 8.6 nm. The transmission electron microscopy (TEM) images of Fe₃O₄ showed that the morphology of magnetite Fe₃O₄ nanoparticle is spherical in shape with uniform grain size and good dispersibility despite the agglomeration it found in some place. The addition of PEG can decrease the agglomeration and reduce the particle size. The existence of PEG layer on Fe₃O₄ was confirmed by Fourier transform infrared (FTIR) spectroscopy. The result of Vibrating Sample Magnetometer (VSM) showed that saturation magnetization (Mₛ) of Fe₃O₄ nanoparticles decreased from 77.16 to 37.15 emu g⁻¹ with the increase of PEG weight from 0% to 50%. Such Fe₃O₄ nanoparticles with favorable size and tunable magnetic properties are promising biosensor applications.

1. Introduction
In recent years, magnetic nanoparticles especially magnetite Fe₃O₄ nanoparticles have been widely used in biomedical applications for collection and separation of bioactive molecules, targeted drug delivery, and biodiagnosis because of its characteristics, such as superparamagnetic, responses to biomolecules, biomolecule-dispersion and high magnetic saturation (Mₛ) [1–3]. To be applied as a biosensor, magnetite Fe₃O₄ nanoparticles must have good dispersibility in aqueous media and narrow size distribution. This is to avoid agglomeration in Fe₃O₄ nanoparticles. However, pure magnetic nanoparticles tend to agglomerate as result of strong magnetic interactions. Therefore, for biomedical applications, one important problem to consider in the use of Fe₃O₄ nanoparticles is stabilization. To increase their stabilization, Fe₃O₄ nanoparticles have been stabilized by the formation of polymer layers on the surface of magnetic nanoparticles, using dextran, gen, cell, poly (vinyl alcohol) (PVA), enzyme, protein, gelatin, etc [4–9].

The use of magnetic nanoparticles to biological applications has two major problems. The first one, as what has been stated previously is easy agglomeration, chemical reactivity and high surface energy, especially when they are used as biological agents without being matched with proper characterization. For this reason, surface modification or coating method of magnetic nanoparticles is required with biocompatible polymer molecules. Polyethylene glycol (PEG) is the most popular and widely used biocompatible polymers because of its benefits such as increasing dispersibility, biocompatible, non-toxic, and low cost.

The co-precipitation method of iron salts is the simplest and most efficient chemical pathway to synthesis magnetic nanoparticle [10]. In this study, PEG-coated Fe₃O₄ was synthesized following a simple two steps co-precipitation approach with various PEG concentrations. The synthesized PEG-coated Fe₃O₄ nanoparticles...
were investigated by x-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, transmission electron microscopy (TEM), and vibrating sample magnetometer (VSM).

2. Experiment details

The synthesis was carried out by chemical co-precipitation method by following the procedure. Synthesis of Fe₃O₄ nanoparticles was obtained from the basic ingredients of dissolved hydrate compounds, namely ferric chloride hexahydrate (FeCl₃.6H₂O) and heptahydrate (FeSO₄.7H₂O) (From Merck, Germany) which are providers of Fe³⁺ and Fe²⁺ ions with a mass ratio of 8.109 g and 4.170 g respectively as shown in figure 1. All the materials had analytical purity and were used without further purification. The dissolution process uses 30 ml of distilled water. It was stirred for 15 min until the mixture is homogeneous. Next, 60 ml of 10% ammonia (NH₄OH) solution was added dropwise and stirred using a magnetic stirrer at a speed of 450 rpm and a temperature of 60 °C for 90 min. The result of the Fe₃O₄ solution was washed using distilled water until the NH₄OH odor disappeared. Next, the Fe₃O₄ decantation process was carried out to obtain samples in the form of sediment. This deposition used the effect of the magnetic field from a permanent magnet to be more efficient. The precipitate is washed 7 times to minimize the amount of salt from other reactions dissolved in the sample [11]. The Fe₃O₄ sample was dried in a furnace for 2 h at a temperature of 80 °C. The relevant chemical reaction can be expressed as follows:

\[
2\text{FeCl}_3 \cdot 6\text{H}_2\text{O} + \text{FeSO}_4 \cdot 7\text{H}_2\text{O} + \text{H}_2\text{O} + 8\text{NH}_4\text{OH} \rightarrow (\text{NH}_4)_2\text{SO}_4 + 6\text{NH}_4\text{Cl} + \text{Fe}_3\text{O}_4 + 24\text{H}_2\text{O}.
\]

PEG-coated Fe₃O₄ was synthesized following a simple two steps co-precipitation approach with various concentrations of polyethylene glycol 4000 (PEG-4000). PEG concentrations are used as in table 1.

The phase identification of Fe₃O₄ and PEG-coated Fe₃O₄ and crystallite size determination were done by x-ray diffractometer Shimazu XD equipped with a crystal monochromator employing Cu-Kα radiation of wavelength 1.5406 Å [12]. The coating and surfactant were only done for observing the functional groups by using Fourier transform infrared (FTIR) spectroscopy [13]. Magnetic characterization was carried out by Riken Denshi Co. Ltd. Vibrating sample magnetometer (VSM) under external magnetic fields up to ±15000 Oe at...
room temperature [14]. The size and morphology were investigated by means of transmission electron microscopy (TEM) using JEOL JEM-1400 [15].

3. Result and discussion

Phase investigation of the crystalline products were conducted using XRD and patterns are presented in figure 2. It is found that the d-spacing values of significant peaks match well with the data from JCPDS card (19-029) for Fe3O4 nanoparticles. The diffraction peaks at 2θ can be assigned to the (220) (311) (400) (511) (440) confirmed the existence of invers spinel [16]. The determination of average crystallite size of Fe3O4 is based on XRD line broadening and calculated using Scherrer’s equation as follows:

$$d = \frac{0.9 \lambda}{\beta \cos \theta}$$

Based on the equation above, d is the average of crystallite size, 0.9 is the Scherrer constant, λ is the x-ray diffraction wavelength (λ = 1.5406 Å), β is the full width at half maximum (FWHM) of plane (311) and θ is the Bragg angle in degree. The estimated diffraction peak is at the angle of 35.4° and the average crystallite size of Fe3O4 is 12 nm and PEG-coated Fe3O4 nanoparticles is 8.6 nm. In addition, another phase appeared on Fe3O4 namely α- Fe2O3 at 32°. This is due to the oxidation process that occurred in the synthesis process [17]. Then there is another phase on PEG-coated Fe3O4 nanoparticles namely γ-FeO (OH), α-FeO (OH), and α-Fe2O3 at 23°, 27°, and 32° respectively. The emergence of new peaks occurred because the Fe3+ atoms that filled the entire tetrahedral position and half of the octahedral on the surface of the nanoparticles reacted with the hydroxyl group (O–H) in PEG. The formation of these peaks indicates the influence of PEG which reacted and functioned as an agent that could limit crystal growth. On the other hand, PEG could also function to reduce oxidation, so the phase of α-Fe2O3 decreased when Fe3O4 nanoparticles were coated with PEG.

The FTIR spectra of magnetite Fe3O4 nanoparticles, polymers polyethylene glycol 4000 (PEG-4000), and PEG-coated magnetite Fe3O4 nanoparticles are shown in figure 3. The Fe3O4 nanoparticles showed dominant absorption peaks associated with Fe-O vibrations at around 620 to 571 cm⁻¹ (figure 3, curve a). The peak of Fe-O absorption in the IR spectrum indicates that the Fe3O4 phase has been formed in the synthesized sample. Each functional group has an infrared absorption band which has the characteristics of a bond at a certain wave number. The vibration of each bond gives an image of a distinctive peak so that it can be used as an identification of a functional group of compounds. Similar peaks were observed in the PEG-coated magnetite Fe3O4 nanoparticles spectra (at 632 and 586.36 cm⁻¹, figure 3, curve c), and observed on PEG-4000 (figure 3, curve b). In addition, the vibration of the O–H (hydroxyl) group is around 2890 cm⁻¹. The appearance of vibrational peaks associated with the O–H group indicates the absorption of water molecules on the surface of Fe3O4 which allows it to bind to the PEG-4000 polymer. A stretch of C–O of about 1105 cm⁻¹ was observed in both PEG and PEG-coated Fe3O4 nanoparticles (figure 3, curves b, c), revealing the presence of PEG residue in the final
product. However, this peak was not observed in the Fe₃O₄ spectrum. These results clearly show the surface modification of Fe₃O₄ nanoparticles with PEG [18].

Figure 4(a) shows the magnetization curve of magnetite Fe₃O₄ nanoparticles at room temperature. The maximum saturation magnetization ($M_s$) gain is 77.16 emu g⁻¹ [19]. The magnetite Fe₃O₄ nanoparticles exhibited good magnetic response. Other than that, it could be easily attracted by the near magnet with superparamagnetic behavior. The magnetization is related to particle size and cation distribution. The remanent magnetization ($M_r$) and coercivity field ($H_c$) gains are 7.65 emu g⁻¹ and 50 Oe respectively. TEM images recorded from Fe₃O₄ nanoparticles and PEG-coated Fe₃O₄ nanoparticles are shown in figures 4(b) and (c) respectively. The morphology of magnetite Fe₃O₄ nanoparticles is spherical with uniform grain size and good dispersibility despite the agglomeration found in some places. The addition of PEG could decrease the agglomeration and reduce the particle size. This is because PEG can modify the surface of Fe₃O₄ particles so that the particle size is more monodisperse and uniform. In addition, lattice fringe patterns for both, are in good agreement with the XRD analysis, which corresponds to the crystal field (220), (311), (400), (440), and (511).

Other information obtained is the grain size distribution of Fe₃O₄ nanoparticles and PEG-coated Fe₃O₄ nanoparticles as shown in figure 5.

Figure 5 shows the grain diameter distribution from TEM observations. The highest frequency shows the average size of grain diameter. The average grain diameter of Fe₃O₄ nanoparticles shown in figure 5(a) is 14.5 ± 0.5 nm whereas from figure 5(b) the diameter of PEG-coated Fe₃O₄ nanoparticles is 12.5 ± 0.5 nm. The good correlation between particle sizes obtained from Scherrer equation and TEM supported the highly crystalline structure as shown by XRD. From the results of the analysis, it can be concluded that PEG is able to control particle size and limit the crystal growth of Fe₃O₄.
Under room temperature, M-H hysteresis of PEG-coated Fe₃O₄ nanoparticles was measured up to 15 kOe using a vibrating sample magnetometer (VSM). As shown in figure 6, all magnetization curves are shaped S-like over an external magnetic field, which is a typical superparamagnetic material, with zero coercivity in the M-H plot. Zero coercivity occurred because there was no residual magnetism when the external magnetic field was removed [20, 21]. The saturation magnetization (Mₛ) of Fe₃O₄ nanoparticles decreased from 77.16 to 37.15 emu g⁻¹ with the increased PEG weight from 0 to 50% [22, 23]. This is also supported by XRD analysis. This phenomenon shows that the greater the size of magnetite Fe₃O₄ nanoparticles, the stronger the saturation magnetization [24]. Thus, the high magnetization indicated that PEG-coated Fe₃O₄ nanoparticles have great potential for biomedical applications [25].

4. Conclusion

In this investigation, Fe₃O₄ nanoparticles have been successfully synthesized by a PEG assisted co-precipitation method in which aqueous NH₄OH solution was used as solvent agents. Formations of PEG-coated Fe₃O₄ nanoparticles are confirmed by XRD and FTIR. TEM analysis confirmed that the physical size and average
crystallite size of the nanoparticles decreased with the increase of PEG weight. The prepared PEG-coated Fe₃O₄ nanoparticles exhibited high saturation magnetization and superparamagnetic behavior which evidence multidomain behavior of the observed particles and the absence of coupling between the nanocrystals due to the presence of the PEG as polymer in the nanoparticles. Since many intrinsic properties of magnetic nanoparticles are size dependent, it can be concluded that the nanoparticles with different sizes will have important application in biomedical applications such as cancer diagnosis, targeted drug delivery, as well as biomolecular separations [26, 27]. Therefore, the high saturation and superparamagnetic behavior of PEG-coated Fe₃O₄ nanoparticle make it promising as biosensor applications.

ORCID iDs

Ganesh Antarnusa https://orcid.org/0000-0002-3592-9333

References

[1] Abu-Dief A M and Hamdan S K 2016 Am. J. Nano Sci. 2 26–40
[2] Abu-Dief A M and Abdel-Mawgoud A H 2018 SFJ Nanochem. Nanotechnol. 1 1005 (https://scienceforecastoa.com/Articles/SJNN-V1-E1-1005.pdf)
[3] Toyos-Rodríguez C et al 2019 Journal of Nanomaterials 2019 2464010
[4] Massia S P, Stark J and Lethbetter D S 2000 Biomaterials 21 2253–61
[5] Edelstein R L, Tamanaha C R, Sheehan P E, Miller M M, Baselt D R, Whitman L J and Colton R J 2000 Biosens. Bioelectron 14 805–13
[6] Pankhurst Q A, Connolly J, Jones S K and Dobson J 2003 J. Phys. D: Appl. Phys. 36 167–81
[7] Wang S, Wang C, Zhang R, Sun Z, Li Z, Jiang X and Bai X 2010 Mater. Lett. 64 9–11
[8] Antarnusa G, Sastika P E and Suharyadi E 2018 J. Phys. Conf. Ser. 1011 012061
[9] Sastika P E, Antarnusa G, Suharyadi E, Kato T and Iwata S 2018 J. Phys. Conf. Ser. 1011 012060
[10] Zhu N, Ji H, Yu P, Niu J, Farooq M U, Akram M W, Udego I O, Li H and Niu X 2018 Nanomaterials 8 810
[11] Loh K S, Lee Y H, Musa A, Salmah A A and Zamri I 2008 Sensors 8 5775–91
[12] Khot V M, Salunkhe A B, Phadatara M R, Thorat N D and Pawar S H 2013 J. Phys. D: Appl. Phys. 46 053303
[13] Phadatara M R, Khot V M, Salunkhe A B, Thorat N D and Pawar S H 2012 J. Magn. Magn. Mater. 324 770–72
[14] Akay S K, Pelşkoz A and Kara A 2018 J. Supercr. Nov. Magn. 31 849–54
[15] Utomo J, Agustina A K, Suharyadi E, Kato T and Iwata S 2018 Intege. Ferroelectr. 187 194–202
[16] Mukhopadhyay A, Joshi N, Chattopadhyay K and De G 2012 App. Mater. Interfaces 4 142–9
[17] Tartaj P, Morales M P, Verdaguer S V, Carreño T G and Serna C J 2003 J. Phys. D: Appl. Phys. 36 182–97
[18] Karaoğlu E, Kavaz H, Baykal A, Toprak M S and Sözeri H 2011 Nano Micro Letters 3 79–85
[19] Gong P, Yu J, Sun H, Hong J, Zhao S, Xu D and Yao S 2006 J. Appl. Polym. Sci. 101 1283–90
[20] Abu-Dief A M, Abdelbaky M S M, Martinez-Blanco D, Amghouz Z and García-Granda S 2016 Mater. Chem. Phys. 174 164–71
[21] Mohamed W S, Alnnaid M, Abdelbaky M S M, Amghouz Z, García-Granda S and Abu-Dief A M 2019 Nanomaterials 9 1602
[22] Akbarzadeh A, Samei M and Davaran S 2012 Nanoscale Res. Lett. 7 144
[23] Wang G, Chang Y, Wang L, Wei Z, Kang J, Sang L, Dong X, Chen G, Wang H and Qi M 2013 J. Magn. Magn. Mater. 340 57–60
[24] Anbarasu M, Anandan M, Chinnasamy E, Gopinath V and Balamurugan K 2015 Spectrochim. Acta A Mol. Biomol. Spectros. 135 536–9
[25] Srivastava V, Gusain D and Sharma Y C 2015 Ind. Eng. Chem. Res. 54 6209–33
[26] Nene A G, Takahashi M and Somani P R 2016 World J. Nano Sci. Eng. 6 20–8
[27] Pelaz B et al 2017 ACS Nano 11 2313–81