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Synthesis and Characterization of Fe₃O₄ Nanoparticles Modified with Polyethylene Glycol as Antibacterial Material

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ABSTRACT

iron oxide (Fe₃O₄) nanoparticles modified with polyethylene glycol (PEG) was synthesized by co-precipitation methods using ferric and ferrous ions as the precursors. Further, the antibacterial activity was performed against gram-positive and gram-negative bacteria. The Fe₃O₄-PEG was characterized using X-Ray Diffraction (XRD), Fourier Transform Infra Red (FTIR), Scanning Electron Microscopy (SEM) with energy dispersive X-Ray analysis (EDAX) and Vibrating Sample Magnetometer (VSM). The particle size of Fe₃O₄-PEG calculated using D is 46.2 nm. The study confirmed that Fe₃O₄-PEG is superparamagnetic and has a saturation magnetization of 56.43 emu/g. The prepared Fe₃O₄-PEG gives the effect of both gram-positive and gram-negative pathogenic bacterial strains hence this material has potential utilization in the field of pharmaceutical and biomedical in the future.

Keyword: Fe₃O₄, nanoparticle, PEG, antibacterial activity

INTRODUCTION

In recent years, spinel ferrite nanoparticles have been the subject of developed research. The dimension of nanoparticle is between bulk materials and atoms or molecules. The spinel ferrite has a structural formula MFe₂O₄, where M is a divalent metal with a cubic spinel crystal structure. In addition, the spinel ferrite has magnetic properties. Magnetic nanoparticles can be used in various applications such as adsorbent [1,2], magnetic storage, ferrofluids, biomedical applications [3,4] and gas sensor [5].

Various methods can be used to prepare magnetic nanoparticles such as co-precipitation [6,7], hydrothermal [8], microemulsion [9], electrochemical route [10,11] and sol-gel [12]. One of the magnetic nanoparticles is Fe₃O₄ (magnetite). The materials exhibit superparamagnetic behavior, low toxicity, biocompatibility, easier surface modification [13,14].

Another study reported that Fe₃O₄ magnetic nanoparticles have antibacterial activity properties. Fe₃O₄ nanoparticles showed strong antibacterial activity, the antibacterial activity caused by the presence of reactive oxygen species (ROS) [4]. The Fe₃O₄ nanoparticle has the greatest antibacterial effect to pathogenic bacteria of *Pseudomonas aeruginosa* than *Escherichia coli* and *Staphylococcus aureus* [15]. In addition, the Fe₃O₄ nanoparticle has a zone of inhibition consideration to Ag nanoparticle for topical use [14].

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Materials in the form of small particle sizes are easy to agglomerate and made it larger particle size, reducing surface area and magnetic properties. Agglomeration can be prevented by coating nanoparticles with organic polymeric materials. The coating process also prevents nanoparticles from oxidation processes, reduces toxicity and increases chemical stability [16,17]. Some research on coating nanoparticles with organic materials such as CoFe₂O₄-alginate [18], Fe₃O₄-polypropylene [8], CoFe₂O₄-chitosan [19], CoFe₂O₄-polyvinyl alcohol, gelatin [16].

In this study, we used co-precipitation method for preparing Fe₃O₄ and coating with polyethylene glycol (PEG)-4000. PEG is long polymer chains with several advantages for coating Fe₃O₄ of non-toxic to a large extent, non-immunogenic, non-antigenic, and protein-resistant polymer [16,20]. In addition, the incorporation of inorganic and organic particles has combination of the properties of inorganic particles such as thermal, mechanical, magnetic, and the properties of organic particles that is flexibility. Furthermore, the Fe₃O₄-PEG were evaluated for antibacterial activities. The bacteria used are gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*).

EXPERIMENT

Chemicals and instrumentation

The chemical reagents used in this study were FeCl₃.6H₂O, FeCl₂.4H₂O, NaOH, HCl and PEG-4000, nutrient agar from Merck. The bacteria species *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 from PT Bio Farma.

Synthesis Fe₃O₄-PEG 4000

The Fe₃O₄ magnetic nanoparticles prepared by co-precipitation. Initially, 5.41 g of FeCl₃.6H₂O and 1.99 g of FeCl₂.4H₂O were added into 20 mL of distilled deionized water. Into the mixture, aqueous solution NaOH 1 M is added dropwise until pH 11 while flowing N₂ gas at room temperature and stirring at 200 rpm [21,22]. The magnetic nanoparticles are black precipitates, which can be separated from the solution using magnet permanent. The powder was washed using distilled water until neutral and then washed using ethanol. The product was dried under vacuum for 3 h at 60 °C. The reaction synthesis of Fe₃O₄ by coprecipitation method is as follows:



The next step was dissolving PEG (2.50 g) in 5 mL deionized water. The solution was stirred for 30 minutes until homogeneous. Then, 0.25 g of Fe₃O₄ was added to the suspension. The mixture was stirred under nitrogen atmosphere for 10 h at 45°C. Fe₃O₄ coated PEG product were separated from the solution by centrifugation. Finally, the product washed with ethanol to dissolve the remaining of PEG.

The crystal structure of Fe₃O₄-PEG was obtained by XRD (Shimadzu XD-3H) with Cu K α ($\lambda = 1.5406 \text{ \AA}$) radiation, magnetic properties were determined by VSM (Lake Shore 7410), in an external field (temperature in the range 10-400 K), functional group was analyzed by FTIR Shimadzu 5400 in the range 4.000-400 cm⁻¹, morphology and element composition of Fe₃O₄-PEG were studied by SEM-EDX JEOL-JSM-6510 LV.

Screening of antibacterial activities

In this study, the test of antibacterial activity was conducted using diffusion disc method [23]. Test bacteria (*Staphylococcus aureus* and *Escherichia coli*) were inoculated to Petri dish with Nutrient Agar (NA) medium, then paper disc with 6 mm diameter were used to inoculated test organism. Fe₃O₄-PEG 10 μL was instilled with different concentration (0; 12.5; 25; 50; 100 and 160 μg/mL). The Petri dish were wrapped by parafilm tape and then all of Petri dish were incubated at 37°C for 24 hours. The antibacterial activities were determined by measurement the zone inhibition diameter in millimeters.

RESULT AND DISCUSSION

Characteristic of Fe₃O₄-PEG 4000

The XRD pattern of Fe₃O₄ and Fe₃O₄-PEG shown in figure 1. The product has a cubic spinel structure in accordance with JCPDS 19-0629. The main peak of Fe₃O₄ that appears on 2θ corresponds to the reflection planes (220), (311), (400), (422), (511) and (440). The Fe₃O₄-PEG spectrum decreased in intensity due to the addition PEG which has amorphous properties. Using the Scherrer formula ($D = 0.89\lambda/\beta \cos \theta$) one can estimate the average size of crystals of nanoparticles, where β is the full width at half maxima (FWHM). Calculation of crystal size based on the peak is highest in reflection plane of the (311) peak. It was found that the Fe₃O₄-PEG crystal size larger than Fe₃O₄. The crystallite sizes is estimated 46.2 and 35.7 nm, respectively. The similar results that Fe₃O₄ nanoparticles coated using polyethyleneimine (PEI) has larger crystal size than Fe₃O₄ without coating [11]. Other studies show that CoFe₂O₄ nanoparticles have a smaller particle size than CoFe₂O₄ that is coated using PEG [24].

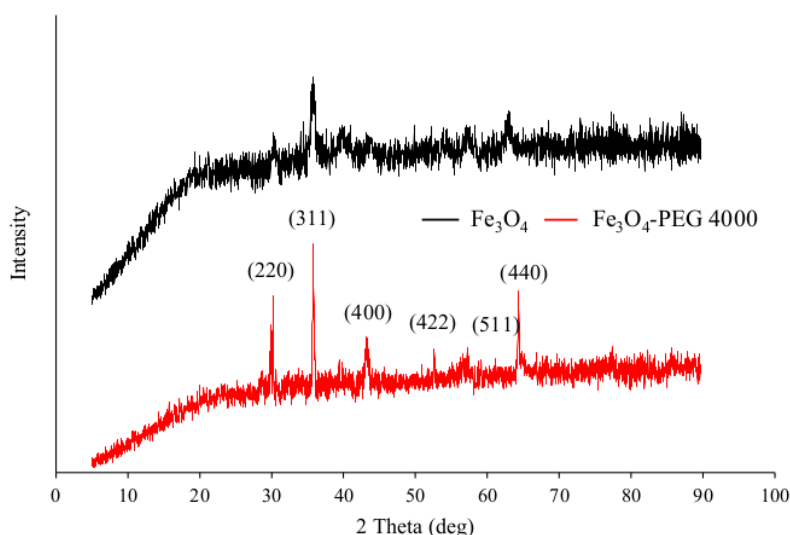


Figure 1. XRD pattern of Fe₃O₄ and Fe₃O₄-PEG 4000.

The presence of PEG on the surface of Fe₃O₄ nanoparticles was evaluated by FTIR. Figure 2 displays the FTIR spectra of Fe₃O₄ and Fe₃O₄-PEG 4000 at range 400-4000 cm⁻¹. The peaks that appeared at the wave number 3390.6 cm⁻¹ on the Fe₃O₄ and 3392.5 cm⁻¹ on

Fe₃O₄-PEG 4000 showed the absorption band of O-H groups from the adsorbed H₂O onto materials. The peaks of Fe₃O₄ and Fe₃O₄-PEG 4000 at the 1626.7 and 1627.8 cm⁻¹ indicate the bending vibrations of O-H. The characteristic peaks of PEG showed at 2862.2 and 1458.1 cm⁻¹ assigned stretching vibration and bending vibration of C-H in -CH₂. The band at 1470 cm⁻¹ is C-C vibration stretching PEG. The peak that appears at 1110.9 cm⁻¹ is to the bond stretching vibrating of C-O [8]. The peak is characteristic of PEG, that does not appear on the Fe₃O₄ spectra. The wave numbers are characteristic of Fe-O bonds shown with a strong peak at 584.4 and 532.3 cm⁻¹ on Fe₃O₄ and Fe₃O₄-PEG, respectively. The shift of wave numbers of Fe-O bond on Fe₃O₄-PEG spectra shows the interaction between Fe₃O₄ and PEG. Several studies have shown that the wave number of the Fe-O bond appears at 569 cm⁻¹ [20], 581 cm⁻¹ [25], and 567.12 cm⁻¹ [10].

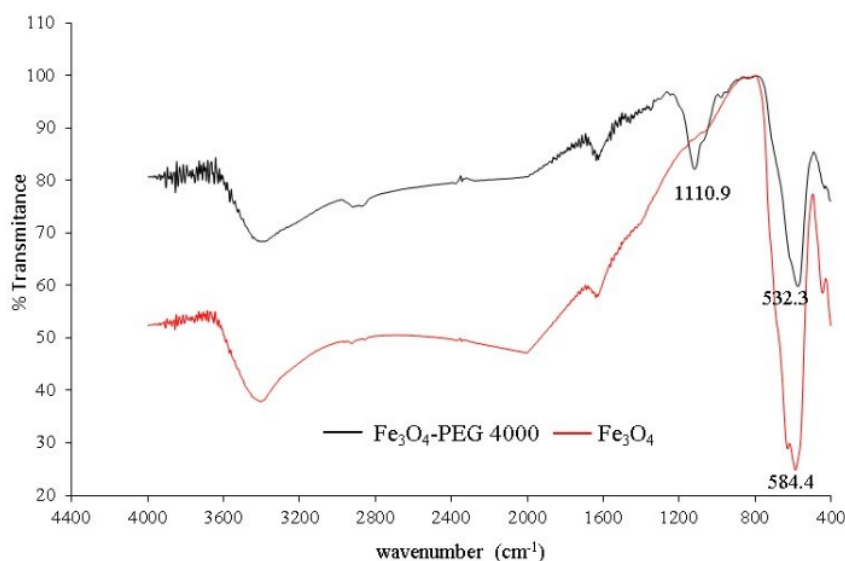
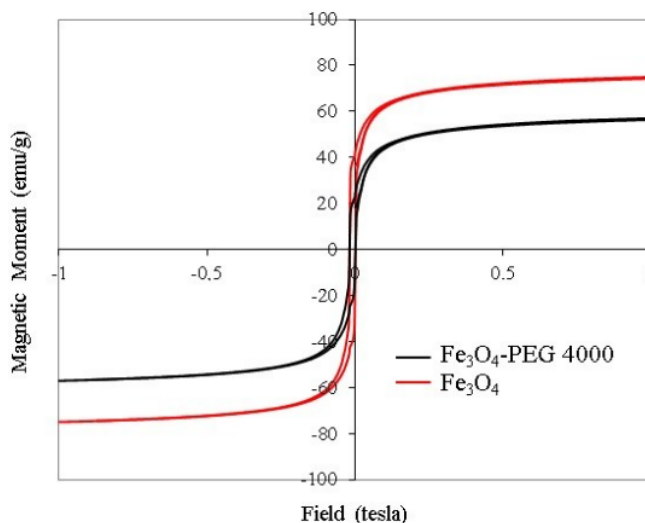


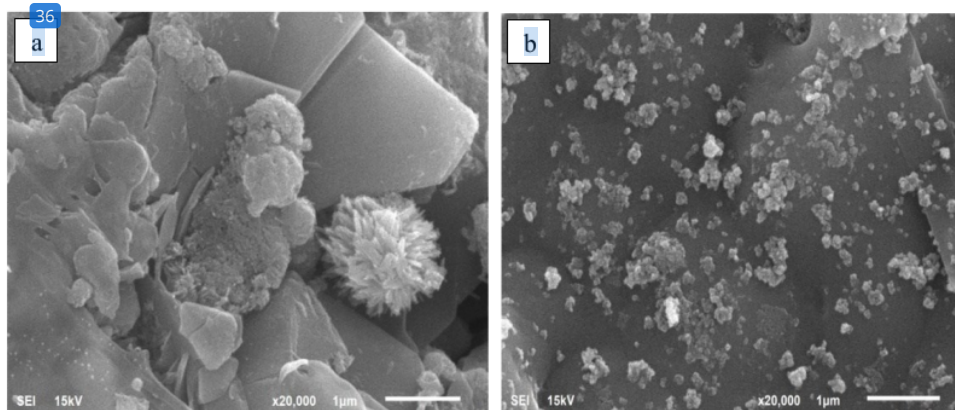
Figure 2. Spectra FTIR of Fe₃O₄-PEG 4000.

Figure 3 shows the magnetic curves of Fe₃O₄ and Fe₃O₄-PEG. It reveals that Fe₃O₄ has high saturation magnetization than Fe₃O₄-PEG. The addition of organic polymers (PEG) causes a small reduction in magnetic properties. Fe₃O₄-PEG is classified as superparamagnetic which in this research shows saturation magnetization of 56.43 emu/g while Fe₃O₄ is 74.33 emu/g. The changes in magnetic properties due to the effect of surface modification of Fe₃O₄ by large polymer molecules. The greater the concentration of PEG is added, the lesser of the saturation magnetization where the polymer coat the nanoparticles so that giving a protection effect from the magnetic field [24]. The saturation magnetization value is similar to the other reference [20,25]. Their study reported Fe₃O₄ modified with sodium citrate and oleic acid with various concentrations of Fe₃O₄ showed magnetization saturation of 50.61 – 61.36 emu/g [25].



5 **Figure 3.** The saturation magnetization of Fe_3O_4 and Fe_3O_4 -PEG 4000.

The morphology of Fe_3O_4 and Fe_3O_4 -PEG 4000 and its constituent elements were analyzed using SEM-EDX. SEM image of Fe_3O_4 and Fe_3O_4 -PEG 4000 are displayed in figure 4. The image shows a clear difference between Fe_3O_4 before and after modification with PEG. The morphology of Fe_3O_4 appears to be agglomerated while modified with PEG appears to be dispersed on PEG surface.



36 **Figure 4.** SEM image of (a) Fe_3O_4 and (b) Fe_3O_4 -PEG 4000

24 Table 1 shows the constituent elements of Fe_3O_4 and Fe_3O_4 -PEG 4000. It can be seen that the addition of carbon elements in Fe_3O_4 -PEG 4000 indicates that modification of Fe_3O_4 -PEG is successful. The main elements of Fe_3O_4 are Fe and O while modified of Fe_3O_4 with PEG affects as the percentage of C element increases.

Table 1. The data of elements Fe₃O₄ and Fe₃O₄-PEG 4000

Materials	Mass (%)		
	Fe	O	C
Fe ₃ O ₄	43.36	56.98	-
Fe ₃ O ₄ -PEG 4000	26.80	38.93	34.27

Antibacterial activity

Antibacterial activities in this study show in figure 5 and table 2. In the figure, we can see that zone of inhibition of Fe₃O₄-PEG 4000 to *Staphylococcus aureus* and *Escherichia coli*. The different concentrations of Fe₃O₄-PEG 4000 give different diameter of inhibition zone. The size of the inhibitory zone depends on the type of bacteria, the size, and concentration of the nanoparticles [26]. The antibacterial activity of Fe₃O₄ can be explained that Fe₃O₄ is positively charged while the bacterium is negatively charged, so there is an interesting attraction between Fe₃O₄ and bacteria. Bacteria is oxidized and dies [4]. In this study, it appears that gram-positive bacteria has a smaller diameter inhibition zone than that in gram-negative bacteria. Another study, also suggest that the gram-negative bacteria are more sensitive compare to gram-positive bacteria [4].

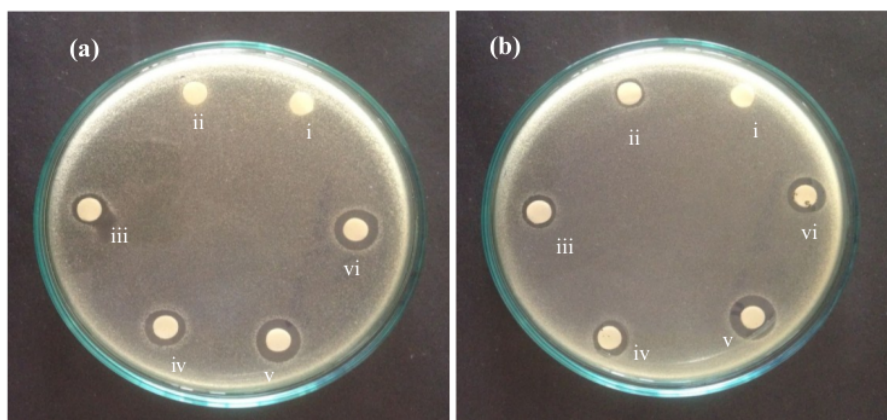


Figure 5. Antibacterial activity of Fe₃O₄-PEG 4000 with concentration (i)0 (ii)12.5 (iii)25 (iv)50 (v)100 and (vi)200 µg/mL to (a) *Staphylococcus aureus* and (b) *Escherichia coli* (give information number in image; the number refer to concentration sample in ppm or other)

The same result was reported previously, that gram-negative have higher susceptibility than gram-positive bacteria. The killing rate of *Escherichia coli* is higher than that in *Staphylococcus aureus* [27]. The differences in susceptibility are caused by differences in cell wall structures, cell physiology and metabolism [15,28]. PEG was also reported to have antibacterial activity. The hydrophilic properties of PEG inhibit bacterial growth. Water is very important for bacteria for growth and multiplication [29]. The MIC (Minimum Inhibitory Concentration) for *Staphylococcus aureus* is 12.5 µg/mL with an average of inhibitory diameter 6.6 mm, and this result is smaller than that in *Escherichia coli* with an average of inhibitory diameter 6.3 mm at concentration 25 µg/mL.

Table 2. The diameter of inhibition zone for Fe₃O₄-PEG 4000

Concentration (µg/mL)	Average inhibitory diameter (mm)	
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
200	11.0	10.2
100	11.1	11.3
50	7.3	8.4
25	6.3	7.2
12.5	0	6.6
0	0	0

CONCLUSION

Fe₃O₄ nanoparticles modified with PEG could be used as an antibacterial material. The Fe₃O₄-PEG showed antibacterial properties on gram-positive and gram-negative bacterial strains. The antibacterial effect of Fe₃O₄-PEG on *Escherichia coli* is stronger than *Staphylococcus aureus*. The MIC value of Fe₃O₄-PEG for *Escherichia coli* is 12.5 µg/mL whilst for *Staphylococcus aureus* is 25 µg/mL. These results suggest that the Fe₃O₄-PEG has a potential application, and further research has to be undertaken for toxicity evaluation in the animal model or human.

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