Antibacterial Performance of Fe₃O₄/PEG-4000 Prepared by Co-precipitation Route

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Abstract: In this paper, we report the synthesis of Fe₃O₄ nanoparticles modifying polyethylene glycol/PEG-4000 by co-precipitation route. The structural and antibacterial features of the samples were studied by means of XRD, SEM, FTIR, and antibacterial test. The results of XRD data showed that Fe₃O₄/PEG was in a spinel cubic structure. The sample presented the combined composition of the Fe₃O₄/PEG-4000 in the nanometric scale in agglomerated form. Interestingly, the Fe₃O₄/PEG-4000 exhibited an excellent performance as an antibacterial agent with the average inhibitory diameter for each Escherichia Coli and Staphylococcus Aureus bacteria are 1.67 mm and 1.97 mm respectively.

Keywords: Fe₃O₄, PEG-4000, co-precipitation route, iron sand, antibacterial agent.

1. Introduction
Nowadays, nanomaterial evokes interests to be studied due to its broad technology applications, particularly magnetic nanoparticles. From various magnetic nanoparticles, magnetite (Fe₃O₄) receives exceptional attention for various biomedical applications, such as drug delivery [1], hyperthermia [2], and magnetic resonance imaging [3] and antifungal material [4]. Besides that, Fe₃O₄ also has high and non-toxic biocompatibility [5], so it is believed to allow access to a promising medical application as an antimicrobial material especially anti-bacterial [6,7]. The previous study has explored Fe₃O₄ as an antibacterial agent caused by reactive oxygen [8,9].

Several methods have been performed to synthesize Fe₃O₄ nanoparticles among them is co-precipitation [10], sol-gel [11], hydrothermal [12], sonochemical [13] and electrochemical route [14]. In general, co-precipitation is the simplest and most economical method to produce the Fe₃O₄ nanoparticles [15]. However, Fe₃O₄ nanoparticles have fast aggregation-prone properties [16]. Therefore, layers or surfactants, which are able to prevent aggregation and stabilize the Fe₃O₄ nanoparticles, are needed as coating agents [17].

Previous work showed that the coating of Fe₃O₄ using polyethylene glycol/PEG could enhance the biocompatibility properties, high adsorption, and chemical stability [18,19]. Furthermore, PEG can increase the biotechnology application because it is more efficient, particularly PEG-4000 [20,21]. Therefore, in the present research, it is essential to prepare the Fe₃O₄ nanoparticles as an antibacterial
agent. Accordingly, this research studied the Fe$_3$O$_4$ nanoparticles which have been modified with PEG-4000 used as an antibacterial agent.

2. Methods

The materials used in this work were iron sand, HCl, NH$_4$OH, H$_2$O, PEG-4000, DMSO, ampicillin, peptone, meat extract, agar, *Escherichia coli* and *Staphylococcus aureus* bacteria. The synthesis was initiated by dissolving iron sand, and HCl stirred using a magnetic stirrer. It is then filtered using filter paper (the sediment was discarded). Therefore, the FeCl$_3$ and FeCl$_2$ solutions were formed. The solutions were reacted with PEG-4000 solution and then titrated with NH$_4$OH using a magnetic stirrer until black sediment was formed. The sediment was washed with H$_2$O until reaching the neutral pH. Next, the sediment was filtered to obtain the Fe$_3$O$_4$/PEG particles. Finally, the filtered sediment was deposited and put in the oven for an hour. An antibacterial testing was performed using a well diffusion method. The first step was making the Fe$_3$O$_4$ test solution dissolved with DMSO. Next, the creation of medium was done utilizing peptone, meat extract, and agar then proceeded with the bacterial hatchery. Then, making well pits and applying bacteria that have been inoculated to the medium surface. After the pit was formed, the test sample was dripped into the well pits. The last stage was observing the sample’s zone of inhibition on *Escherichia coli* and *Staphylococcus aureus* bacteria.

3. Results and Discussion

From Figure 1, it can be seen the results of the X-ray diffraction from Fe$_3$O$_4$/PEG nanoparticles. In relation to the phase formation, Rietica software was utilized to identify the success of this synthesis, and the results were plotted according to Figure 1. Based on above diffraction pattern, a peak appears characterizing Fe$_3$O$_4$ namely at $2\theta = 30.3^\circ$, $35.4^\circ$, $43.3^\circ$, $53.7^\circ$, $57.3^\circ$, $62.9^\circ$, and $74.3^\circ$ which are compatible with ($hkl$) plane of (022), (113), (004), (224), (115), (044), and (533) where the highest peak was at the angle of ($2\theta$) $35.4^\circ$ on ($hkl$) plane (113). Through the highest peak, it can be found out the particle size by calculating FWHM ($\beta$) value from the peak of the diffraction plane. It is where the average size of the crystal is determined by the expansion of X-ray diffraction peaks using Scherrer formulation according to Equation 1 [22].

![Figure 1. The diffraction pattern of Fe$_3$O$_4$/PEG-4000 nanoparticles and its refinement result](image-url)
\[ D = \frac{k \lambda}{\beta \cos \theta} \]  

where  
\( k = \text{Scherrer constant} \ (0.9) \)  
\( \lambda = \text{Wavelength} \ (\text{nm}) \)  
\( \beta = \text{FWHM} \ (\text{radiant}) \)  
\( \theta = \text{Bragg angle} \ (\text{radiant}) \)

Based on the analysis of the Fe\(_3\)O\(_4\) highest peak expansion, it was obtained the highest peak expansion was at the angle of \((2\theta) 35.44^\circ\) and the FWHM value was 1.88 rad. Thus, according to Equation 1, the crystal size obtained was 13 nm. This value is not significantly different from the crystal size from the refinement result. On the previous report, Kanagasubbulakshmi \textit{et al.} conducted a Fe\(_3\)O\(_4\) synthesis with the assistance of natural materials with a sol-gel method and obtaining a bigger particle size, namely in the range between 14 nm to 18 nm [23]. Another report, Chen \textit{et al.} performed Fe\(_3\)O\(_4\) particle synthesis using a hydrothermal method and obtaining a diameter of 30 nm [24]. Therefore, the coprecipitation method is proven effective in synthesizing the magnetic nanoparticle with a relatively small size. The shape of the crystal structure of Fe\(_3\)O\(_4\) was inverse spinel cubic which can be observed using the diamond software as seen in Figure 2.

**Figure 2.** The Fe\(_3\)O\(_4\) crystal structure

Based on Figure 2, the green-colored atom represents Fe atom forming the tetragonal site, the blue-colored atom represents Fe atom forming the octahedral site, and the red-colored atom represents O atom. All Fe\(^{2+}\) ions occupied half of the octahedral site and Fe\(^{3+}\) is shared equally to the rest of octahedral and tetrahedral sites [25]. The crystal structure of Fe\(_3\)O\(_4\) is inverse-spinel cubic [26] which shows the structural differences with other Fe derivative compounds such as maghemite (cubic with a space group of \(P4_132\) [27] and hematite (rhombohedral) [28]. This is also affected by reaction stages of Fe\(_3\)O\(_4\) formation as seen in Equation 2-4 [29]:

\[
\begin{align*}
\text{Fe}^{2+} + 2(\text{OH})^- & \rightarrow \text{Fe(OH)}_2 \quad (2) \\
3\text{Fe(OH)}_2 + \frac{1}{2}\text{O}_2 & \rightarrow \text{Fe(OH)}_3 + 2 \text{FeOOH} + \text{H}_2\text{O} \quad (3) \\
\text{Fe(OH)}_3 + 2 \text{FeOOH} & \rightarrow \text{Fe}_3\text{O}_4 + 2 \text{H}_2\text{O} \quad (4)
\end{align*}
\]
Figure 3. (a) The morphology and (b) size distribution of Fe$_3$O$_4$

Figure 3 shows that the uniformity of Fe$_3$O$_4$/PEG nanoparticles and Fe$_3$O$_4$ nanoparticles has been achieved. This nanoparticle has the average size of 17 nm. Nanoparticle consists of fine particles with regular ball morphology with a little agglomeration. The agglomeration phenomenon is a natural tendency owned by Fe$_3$O$_4$ nanoparticle due to the Van der Waals force and also the pulse of a magnetic dipole. Therefore, the nanoparticles form the distributed cluster with the most dominant size which is around 17 nm. Visually, it can be seen that there is a white-colored particle which represents PEG 4000 used in Fe$_3$O$_4$ synthesis. This shows that Fe$_3$O$_4$ nanoparticle is well bound with PEG 4000 and becomes a proof that the coprecipitation method is efficient in the synthesis of Fe$_3$O$_4$/PEG 4000 nanoparticle.

Figure 4. The Functional groups of Fe$_3$O$_4$/PEG

Figure 4 shows the FT-IR spectrum from Fe$_3$O$_4$/PEG. The main functional group which characterized the existence of Fe$_3$O$_4$ located in the area of 416.4 cm$^{-1}$ represents Fe atom in tetragonal site and in the area of 568.4 cm$^{-1}$ for Fe atom on octahedral site spinel structure [30]. The peak on the area of 1613.7 cm$^{-1}$ and 3400.5 cm$^{-1}$ shows there is an OH bond accompanying the formation reactions using a
coprecipitation method [31]. Furthermore, there are several peaks characterizing the functional group of PEG-4000, namely in the area of 1174.6 cm\(^{-1}\) which is a C-O bound, the peak on the area of 1402.2 cm\(^{-1}\) and 2887.1 cm\(^{-1}\) which represents the existence of COO\(^-\) bound [32–34]. Through the characterization using the above FTIR, it can be claimed that between Fe\(_3\)O\(_4\) and PEG-4000 have a bound both through a synthesis process of coprecipitation.

![Figure 5. The antibacterial activity on E. coli and S. aureus bacteria](image)

The antibacterial testing used a well diffusion method and the bacteria used were *Escherichia coli* and *Staphylococcus aureus*. The well diffusion method showed the diameter of the antibacterial inhibitory zone. The antibacterial testing results shown in Figure 5 indicate that Fe\(_3\)O\(_4\)/PEG-4000 has the inhibitory power on Escherichia coli and Staphylococcus aureus bacteria. From the testing result, it also shows that Fe\(_3\)O\(_4\)/PEG-4000 has potentials in inhibiting *Escherichia coli* and *Staphylococcus aureus* bacteria. In the previous research, Sheikh *et al* show the antibacterial results for ferrofluid samples with Fe\(_3\)O\(_4\) filler, with the same rate of Fe\(_3\)O\(_4\) as in this research, the results that they obtained is approximately 1.7 mm [35]. In this study, Fe\(_3\)O\(_4\) which was modified by PEG-4000 has the inhibitory power of 1.67 and 1.97 for *Escherichia coli* and *Staphylococcus aureus* bacteria respectively. However, the increased performance of Fe\(_3\)O\(_4\) is still relatively low since it only relies on the ability of reactive oxygen species which is contained inside it [36]. Therefore, for the future research, a performance improvement of Fe\(_3\)O\(_4\) nanoparticle needs to be conducted from the aspect of structural modification both with the doping [37] and nanocomposite techniques [38].

### 4. Conclusion

The size of Fe\(_3\)O\(_4\) nanoparticle modified by PEG-4000 was 12.21 nm. Fe\(_3\)O\(_4\) nanoparticles with a spherical shape experienced an agglomeration with the size of 17 nm. Fe\(_3\)O\(_4\)/PEG-4000 has potentials in inhibiting *Escherichia coli* and *Staphylococcus aureus* bacteria. However, Fe3O4/PEG-4000 is more effective in inhibiting the growth of *Staphylococcus aureus* bacteria. The average numbers of inhibitory power are 1.67 nm and 1.97 nm respectively.
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