Preparation of Fe₃O₄/OA/DMSO Ferrofluids using a Double Surfactant System as Antifungal Materials Candidate

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Abstract. This research aimed at identifying the structure and potentials of Fe₃O₄ coated with a double surfactant system as an antifungal material. The double surfactant was maintained by exploring dimethyl sulfoxide (DMSO) and oleic acid (OA). The samples were varied with temperature treatments at room temperature until 200 °C. Based on the results of XRD data analysis, it was found that the sample phase was Fe₃O₄ overall with the highest peak at 35.59° and the particle size of approximately 10 nm. Based on the SEM-EDS characterization, the morphology of the samples and the constituent element of Fe₃O₄ could be identified; they were Fe and O with the mass percentage of each sample was 78.7% and 21.3% respectively. FTIR was used to identify the sample functional group and was able to detect the existence of constituent component of Ferrofluid which was Fe₃O₄ at wavenumbers of 596 and 713 cm⁻¹, then the stretching bonds of oleic acid were at the wavenumbers of 1312, 1376, 1464, 2856, and 2920 cm⁻¹, DMSO at the wavenumbers of 954 and 1018 cm⁻¹, and the stretching bonds of olive oil at the wavenumbers of 1089, 1169, 1233, 1599, 1742, 2856, 2920, and 3008 cm⁻¹. The antifungal potential was tested using a diffusion method and the best result was obtained for the sample treated at room temperature with the fungal zone of inhibition was around 13 mm and potentially recommended for an antifungal material.

Keywords: Ferrofluid, Fe₃O₄, OA, DMSO, antifungal

1. Introduction
The development of smart material based on Fe₃O₄ has a vast improvement moreover in the study of its properties and benefits in various fields. One of the developed smart materials was Fe₃O₄ ferrofluid. Ferrofluid is in great demand by the researchers because it has a uniqueness compared to other materials such as being able to maintain its condition like liquid substance even though being put in a high external magnetic field [1]. Nowadays, ferrofluid is often used in various applications in the developed technology field and medical fields such as MRI [2], hyperthermia therapy [3], drug delivery system [4], antibacterial [5], and antifungal [6].

In this research, Fe₃O₄ ferrofluid was based on natural materials and then developed as an antifungal material candidate. This is because Fe₃O₄ as filler on ferrofluid has potentials to bind the function of the cell wall; therefore there was a membrane disturbance which led to prevention of the fungal reproduction.
In addition, the antimicrobial activity from reactive oxygen species (ROS) could also destroy DNA and protein in the pathogen organism such as fungus [6,7]. Furthermore, the previous research results showed that nanomagnetic particles coated with biocompatible molecules and treated with back and forth movement of the magnetic field could trigger a superparamagnetic heating phenomenon. Thus, there was a formation of fungal target-lock interaction on the cell surface [10].

In order to achieve a high performance as an antifungal material for Fe₃O₄ ferrofluid, one of the aspects that needs to be fulfilled is the selection of an appropriate surfactant for a good and long-term stability. This step is important to be concerned since the ferrofluid performance was strongly affected by ferrofluid stability. The research results obtained by Kurimsky et al. showed that ferrofluid coated with surfactant shows a good stability, but it is not durable for a long period. This was proven by testing ferrofluid in the thermal aging and showed there was a dividing phase indicating the in-homogeneity on ferrofluid [11].

Based on the elaboration above, it is very important for a double-surfactant coating technology to be further developed. This aims to generate ferrofluid with better stability and longer durability for the application in the medical field. In this research, the first surfactant used was oleic acid (OA) due to its advantages which can minimize magnetic nanoparticle agglomeration prior to the application in the medical field [12]. Further, the second surfactant used as the double surfactant was dimethyl sulfoxide (DMSO). This surfactant was used with the objective of improving the filler stability which is already covered by oleic acid as the first surfactant and enhancing its biological performance [13]. Specifically, this research studied the structural characteristics and antifungal on Fe₃O₄ ferrofluid.

2. Methods
In this research, the basic material used was natural sand taken from Sine Beach, Indonesia. The natural sand was washed and dried for a day, and then separated using a permanent magnet to separate the iron sand from the impurity. The obtained iron sand was reacted with HCl and followed by the reaction with NH₄OH in a sonicator for 30 minutes to generate Fe₃O₄ particle deposits. Each 1 gram of Fe₃O₄ deposits was mixed with 2 mL oleic acid as the first surfactant layer and then heated at varied temperatures namely at room temperature, 100, 150, and 200 °C with the codes for each temperature were FF1, FF2, FF3, and FF4. After that, the mixture was recoated by the second surfactant with 2 mL of DMSO stirred with a magnetic stirrer for an hour and lastly dispersed in the olive oil. The four samples’ crystallinity was tested using XRD, its morphology using SEM, its functional group using FTIR, and its ability to inhibit the growth of Candida albicans fungus was through diffusion method.

3. Results and Discussion
The results of XRD characterization for Fe₃O₄ particles synthesized from the natural sand of Sine Beach Tulungagung and coated with oleic acid as the first surfactant are presented in Figure 1. The fitting process used the data model from ICSD 36314 and marked with red color. Based on the above testing results, it was known that the highest peak was at 35.59° with (h k l) plane (3 1 1) and the distribution of particle size of 10.4 nm; these results have a value approaching the previous study [14]. On the previous report, Arachige et al. stated that Fe₃O₄ nanoparticles have been successfully synthesized using a commercial material with the size of 11.7 nm [15]. Stated in another report, Fe₃O₄ nanoparticle size result which was also synthesized using a commercial material, Panda et al. obtained a result of 12.6 nm [16]. Based on the comparison with the aforementioned reports, the natural material used in this study has a relatively smaller size and appropriate to be synthesized to become Fe₃O₄ nanoparticles.
Figure 1. The XRD pattern of the Fe₃O₄ particles

Besides Fe₃O₄ nanoparticles, the ferrofluid which was successfully synthesized was also characterized using XRD. The sample was dried at a temperature of 200 °C for 3 hours and generated results as shown in Figure 2a. The obtained results for the four samples showed an amorphous phase. This was caused by the filler Fe₃O₄ coated with two surfactants and dispersed in the olive oil. Therefore, during the drying process, filler Fe₃O₄ ferrofluid was mixed with the surfactant and the more dominant number of the dried carrier medium. Interestingly, at the position of 35.6°, there was a peak with (3 1 1) identified (h k l) plane. If a comparison is performed, the XRD results of powder Fe₃O₄ have the highest peak at the value around 90 for intensity and this peak emerged as the Fe₃O₄ representation on the XRD results of dried ferrofluid. When further reviewed, in the process of sample preparation for dry ferrofluid characterization, all ferrofluid components were heated until relatively dry and then smoothened using a mortar. This particular process causes the sample to change into amorphous. This is similar to the research conducted by Mishra and Nayar, where they characterized ferrofluid coated with collagen as a surfactant [17]. And then, the limitation of XRD also becomes the affecting factor because XRD can only diffract atoms on the sample surface [18], where the sample surface was a surfactant, not the filler Fe₃O₄, even more, the filler used has a nano size and the atoms are hard to be detected. For such case, the researchers suggested analyzing the hierarchy of ferrofluid nanostructure with the small angle scattering instrument [19].

The amorphous condition of ferrofluid was strengthened by the results of SEM data in Figure 2b. The dried ferrofluid sample seemed to agglomerate and tended to have a bigger size. The increase of this size was due to the heating process in the stage of sample preparation. If reviewed further, there were whiter parts compared to others. This part was the nonconductive ferrofluid component in the scanning process. Therefore, it can be identified that the part was surfactant covering the filler [20]. However, in this research, it cannot differentiate the SEM results between the first and second surfactant [21]. The researchers suggest using HR-TEM for characterization so that the information of results obtained is more accurate. However, the element inside the sample was also identified using EDS. The dominant elements obtained were Fe and O which were the main constituent elements of filler Fe₃O₄ with the mass percentage of Fe was 78.7% and O was 21.3%. Bonyasi et al. analyzed the Fe₃O₄ used as a filler in the core-shell having a lower percentage of Fe element [22]. In this article, the researchers provide brief and basic information related to EDS characterization so that bias did not occur. Even
though EDS was a characterization integrated with SEM, principally they are both different. EDS is able to identify the element in the sample by detecting the dispersed sample energy. Therefore, the element in the sample can identify clearly.

The sample functional group was tested using FTIR to confirm the existence of all constituent components of ferrofluid. The FTIR results of four ferrofluid samples were shown in Figure 3. Based on Figure 3, it can be found out in the four samples that the Fe-O vibration signifies the emergence of Fe$_3$O$_4$ in the area of 596 and 713 cm$^{-1}$ [22]. Additionally, there were peaks showing the appearance of oleic acid as the first surfactant. The peaks were the double bound C=C peak which was the characteristic of oleic acid appeared on the areas of 1312 and 1376 cm$^{-1}$, and then there was a peak representing the bond of a carboxyl group (COO$^-$) existed in the area of 1464 cm$^{-1}$[23]. Next, the CH$_2$ bond in the areas 2856 and 2920 cm$^{-1}$[17,18]. With the existence of those bounds, it can be confirmed that the vibration of carboxyl and C-H bound thoroughly has successfully shown the existence of oleic acid and can bind well with magnetic nanoparticles in its role as a surfactant.

Figure 2. a) The XRD patterns of dried four ferrofluid samples b) The SEM image of dried selected ferrofluid sample FF1

Figure 3. The FTIR spectra of four ferrofluid samples
The next constituent was DMSO as the second surfactant. There was one typical bound which signifies the existence of DMSO, which was S=O bind identified in 954 and 1018 cm\(^{-1}\) [19,20]. The last component was olive oil as the dispenser medium with the functional group namely C-H bound which was conjugation bound in 1089 cm\(^{-1}\), ester (CO) bound in the area of 1169 and 1233 cm\(^{-1}\), ester carbonyl (C=O) in 1599 and 1742 cm\(^{-1}\) and mainly was the bond of unsaturation of olive oil in 3008 cm\(^{-1}\) [28]. The next interesting thing was the existence of symmetrical and asymmetrical CH\(_2\) in the area of 2856 and 2920 cm\(^{-1}\), which also contained by oleic acid [29]. If studied further, the functional group signified oleic acid also represented olive oil because the content of oleic acid in olive oil was very high, ranged between 55 to 83% followed by other unsaturated fatty acids such as palmitate acid and linoleate acid with the percentage of \(~\)20% [30]. Based on this fact, the selection of olive oil as the ferrofluid dispenser medium with oleic acid and DMSO as surfactants was already appropriate.

The testing results of ferrofluid samples to find out the inhibition power on \textit{Candida albicans} fungus using the diffusion method are shown in Figure 4. Based on Figure 4, the clear zone representation the \textit{Candida albicans} fungal zone of inhibition was measured using calipers and presented in Figure 5. In Figure 5, it can be seen that the sample’s zone of inhibition experiencing a decline along with the increase of the varied temperatures applied. The best result was FF1 which was a sample without a heating, while the lowest was FF4 which was given a heating treatment at a temperature of 200 °C. However, the FF4 sample still has a better inhibiting power compared to the negative control. If examined further, increasing the heating temperature will affect significantly on the structure of filler ferrofluid. The nanomagnetic particles heated over the temperatures of 100-150 °C will experience a change in the structural phase of Fe\(_3\)O\(_4\) becomes γ-Fe\(_2\)O\(_3\) due to the oxidation process which eventually affecting on the increase of sample particle size [25], [26]. The correlation with sample antifungal performance due to the oxidation process has caused the reactive oxygen species to lessen. Accordingly, the ability of the sample to break the DNA and protein in the fungus weakened. This what caused the sample zone of inhibition declines along with the rise of the temperature [33]. However, if seen from its stability, the four samples which were successfully synthesized did not show any separating stage meaning that it has a good homogeneity. During the observation process for 4 days, the used samples showed a consistent result, this is important in the biomedical application, the consistent stability is important to achieve.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{The results of antifungal testing on four ferrofluid samples}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5.png}
\caption{The antifungal testing results histogram of four ferrofluid samples}
\end{figure}
4. Conclusion
The synthesis of double surfactant Fe₃O₄ ferrofluid has been successfully performed. Based on the XRD data analysis results, the samples had a Fe₃O₄ phase with the highest peak in the 35.59° and had the particle size of 10.4 nm. Based on the SEM-EDS results, it can be revealed the morphology of amorphous sample and the dominant element on the sample were Fe and O which was the main constituent element of magnetic nanoparticles with the mass percentages of Fe was 78.7% and O was 21.3%. The functional group was characterized using FTIR and three main constituents of ferrofluid were identified to bind well. The result of *Candida albicans* antifungal testing using a diffusion method showed the increase of sample heating temperature affects the antifungal performance. This was in the form of declining antifungal activities due to the oxidation process during the heating. The best result showed in sample FF1 which was prepared at room temperature with the zone of inhibition was approximately 13 mm and these results can be suggested for an antifungal material candidate.

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**Acknowledgments**

We would like to thank the KEMENRISTEKDIKTI for providing “PDUPUT” research grant for the 2017-2018 academic year for AT.