The role of pH in the biosynthesis of Fe₃O₄ nanoparticles prepared from Fe(III) nitrate and matoa leaf extract

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Abstract. Research on nanoparticles is becoming a matter of great interest with constant developments. In particular, biosynthesis is attracting a great deal of attention as an eco-friendly and economical method to obtain nanoparticles. In this report, magnetite (Fe₃O₄) nanoparticles are biologically synthesized using matoa leaf extract (Pometia pinnata) as reducing agent and Fe(NO₃)₃·9H₂O as precursor. The biosynthesis was performed under different pH conditions, that is, 7, 9, and 11, and the as-prepared nanoparticles were characterized using UV–vis, TEM & Selected Area Electron Diffraction (SAED), particle size analyzer (PSA), and XRD analyses. The results obtained from the TEM analysis indicate that the smallest nanoparticles, with sizes ranging from 5 to 10 nm, were obtained at pH 11, which was supported by the PSA result. The TEM result also shows that the nanoparticles became agglomerated, and their sizes were rather diverse. The XRD result showed that the crystallinity of the nanoparticles improved with the basicity of the solutions. From the characterization of the biosynthesized nanoparticles, it was concluded that the smallest nanoparticles exhibiting the best crystallinity were found under pH 11 conditions.

1. Introduction

During the last few years, the field of nanoparticles has attracted growing interest from researchers. Nanoparticles, which have typical sizes in the range 1–100 nm, can come in the form of metals, metal oxides, semiconductors, polymers, carbon materials, organic compounds, or biological compounds such as proteins, DNA, or enzymes. The tiny size of nanoparticles makes them more reactive to their surroundings [1]. In particular, iron oxide magnetic nanoparticles have been proven to be useful in different fields, including electronics, energy, catalysis, and medicine [2]. On this account, the development of methods for the synthesis of iron oxide magnetic nanoparticles constitutes an active area of research.

Biosynthesis provides an attractive alternative to physical or chemical synthesis. In such methods, organisms, for example, plants, or microorganisms are used as environment-friendly reducing agents in the synthesis of nanoparticles [3]. Because the biosynthesis that involves microorganisms is relatively more expensive than that based on plant extracts, the latter can be envisaged as a viable eco-friendly and economical method to synthesize nanoparticles [4].

Biosynthesis has been extensively used for the preparation of silver, gold, palladium, and iron oxide nanoparticles [5]. Among these, iron oxide nanoparticles such as magnetite (Fe₃O₄), maghemite (γ-Fe₂O₃), and hematite (α-Fe₂O₃) have become a matter of great interest because of their unique magnetic properties on nanoscale, which render them useful in numerous applications, such as catalysis, magnetic storage device, and contrast agents in MRI, biosensor, and immunomagnetics [6].
In this research, the synthesis of magnetite (Fe₃O₄) nanoparticles was done biologically using matoa leaf (Pometia pinnata) extract as reducing agent and Fe(NO₃)₃·9H₂O as precursor. For the optimization of the biosynthesis parameters, three biosynthetic solutions with different pH values (7, 9, and 11) were prepared. The biosynthetically prepared nanoparticles were characterized using UV–vis, Transmission Electron Microscopy (TEM) and Selected Area Electron Diffraction (SAED), particle size analyzer (PSA), and XRD analyses.

2. Materials and methods

2.1. Materials
Matoa leaf (Pometia pinnata) was obtained from Universitas Indonesia plant collection. After being cleanly washed, leaves were left to dry for 1 day at room temperature and dried further by oven heating at 40 °C. The dried leaves were then blended to powder. The precursor Fe(NO₃)₃·9H₂O was obtained from Emsure, and the base conditioner (NaOH) and ethanol for the purification of nanoparticles were obtained from Merck. The chemicals were used without further purification. Aquabides was used for the preparation of the solutions in the biosynthesis of nanoparticles.

2.2. Preparation of nanoparticles
Matoa leaf (4 g) was put into 400 mL of aquabides and then heated at 80 °C for 40 min. under magnetic stirring. The extract solution was then filtered using Whatmann filter paper no. 1 and stored in the refrigerator for further uses. The precursor solution was prepared by dissolving 3.232 g of Fe(NO₃)₃·9H₂O powder in 800 mL of aquabides. NaOH powder was dissolved in 100 mL of aquabides to prepare a 1 M NaOH solution. For the biosynthesis, three different solutions with pH values of 7, 9, and 11, respectively, were prepared as follows: three solutions containing the precursor solution and the extract solution in a 2:1 volume ratio were prepared. Then, NaOH drops were added until the pH values became 7, 9, and 11, and the resulting solutions were magnetically stirred at 500 rpm for 2 h at 25 °C. Next, the solutions were left to cool down to room temperature. The obtained nanoparticles were washed using 100% ethanol three times.

2.3. Characterization of nanoparticles
In general, characterization of nanomaterials provides information on their physical and chemical properties. In this research, spectrophotometry was performed using a Thermo UV–vis 105 Genesys spectrometer to investigate the optical properties of the nanoparticles. The measurements were conducted at 1 nm resolution in the 200–800 nm wavelength range. For the analysis of the morphology, crystal structure, and specimen composition of the nanoparticles, a TEM FEI Tecnai G20 S Twin 200 kV microscope was used, and their size distribution, uniformity, and colloidal stability were analyzed using a Zetasizer Nano Series Malvern Particle Size Analyzer with alumina as crucible. XRD Rigaku Smartlab 3 kV diffractometer was used to investigate the crystal structure and phase of the biosynthetically produced nanoparticles. Prior to this latter analysis, the samples were annealed at 250 °C for 3 h. The XRD measurement result was then compared with JCPDS data.

3. Results and discussion

3.1. Absorbance spectrum of iron oxide nanoparticles
Monitoring of the biosynthetic reactions was done by visual examination, in which solution color change indicated that the reactions occurred [7]. Figure 1 and figure 2 show that the color of the solutions changed from yellow, brown, and transparent for the precursor, extract, and NaOH solutions, respectively, to black after 2 h of biosynthesis. This color change indicated that the biosynthesis of iron oxide nanoparticles had occurred. A similar result was found by Martínez-Cabanas et al. [8], who synthesized iron oxide nanoparticles using Eucalyptus globulus leaf extract.

The optical properties of the materials were investigated on the basis of their UV–vis absorbance spectra. Sundaram et al. [9] conducted the biosynthesis of iron oxide nanoparticles using Bacillus subtilis bacteria. In their experiment, the nanoparticles gave rise to UV–vis absorbance peaks at 250–300 nm wavelength. Figure 3 shows the absorbance spectra of the three tested samples. In all samples, a maximum light absorbance appeared at the ultraviolet area around 280–300 nm wavelength.
Figure 1. Solution color of components (a) extract, (b) precursor and (c) NaOH for the preparation of iron oxide nanoparticles.

Figure 2. Solution color after 2 h of biosynthesis in (a) pH=7, (b) pH=9 dan (c) pH=11.

Figure 3. UV–vis spectra of the biosynthetically produced solutions.

Figure 4. (a) TEM and (b) SAED images of the biosynthetically produced pH 7 solution.

3.2. TEM and SAED
Figure 4a displays the TEM result of the pH 7 solution, where round and agglomerated nanoparticles can be seen. The sizes of the nanoparticles were not uniform, ranging from 10 to 20 nm. In figure 4b, the SAED pattern of the pH 7 solutions is shown, in which the white ring pattern is indicative of the polycrystal properties.
On the other hand, the TEM result of the pH 9 solutions shows that the nanoparticles were not uniform in shape, as can be seen in figure 5a. These nanoparticles appeared to be round and agglomerated, with sizes ranging from 30 to 50 nm. The corresponding SAED pattern in figure 5b shows that the white ring pattern was less intense in this case, which is indicative of the formation of polycrystals with weak crystallite properties.

For the pH 11 solution, scratch patterns and tiny black dots were observed in the TEM image (figure 6a), which are characteristic of nanoparticles sized 5–10 nm. The shape and size of the nanoparticles were not uniform, and they were agglomerated. Again, the SAED pattern confirmed their polycrystallinity, as can be seen in figure 6b.

3.3. PSA and zeta potential
The results of the TEM analysis showed that the three solutions produced agglomerated nanoparticles with diverse sizes and shapes. Prasad et al. [10] conducted the biosynthesis of Fe₃O₄ nanoparticles using pea pod extract, finding that the resulted nanoparticles were agglomerated because of the presence of hydroxyl groups from the plant extract. In our case, the nanoparticles were subjected to PSA and ZP tests to confirm whether they were agglomerated.

PSA was used to ascertain the average size and colloidal stability of the produced nanoparticles. The three solutions were found to produce relatively large nanoparticles in average. The average particle sizes were 988, 1325, and 410 nm for the pH 7, 9, and 11 solutions, respectively. The large size of the nanoparticles found in the PSA analysis was in line with the results obtained from the TEM analysis, which showed that the nanoparticles experienced agglomeration.
Figure 7. XRD results of the three biosynthesized samples

The uniformity of the particle size can be determined from the PDI values obtained in the PSA test. Thus, PDI values ≤ 0.1 are indicative of highly monodisperse nanoparticles, PDI values ranging from 0.1 to 0.4 suggest the presence of moderately polydisperse nanoparticles, and highly polydisperse nanoparticles are indicated by PDI values > 0.4 [11]. We found that the PDI values of the pH 7, 9, and 11 solutions were 0.8, 0.6, and 0.56, respectively, which suggests that the produced nanoparticles were not uniform in shape. This finding supported the TEM result.

A zeta potential analysis was conducted to understand the colloidal stability of the nanoparticles. In general, a zeta potential (ZP) value ranging ±0–10 mV is characteristic of unstable nanoparticles, ±10–20 mV of relatively stable particles, ±20–30 mV of stable nanoparticles, and more than ±30 mV of highly stable nanoparticles [11]. Because the ZP values of the pH 7, 9, and 11 solutions were 2.83, −18.8, and −10, respectively, it can be concluded that the nanoparticles obtained from the pH 7 solution were highly unstable and those corresponding to the pH 9 and 11 solutions were unstable.

3.4. XRD

The phase and crystallinity of the nanoparticles was investigated using XRD analysis. As can be seen in figure 7, intensity peaks occurred for the three samples, albeit of relatively low intensity. These results were compared with previously reported XRD results. In the pH 7 solution, two intensity peaks appeared at 2θ 35° (311) and 63° (511). On the other hand, five intensity peaks were observed in the XRD spectrum of the pH 9 solution at 2θ 31° (220), 35° (311), 42.5° (400), 52.5° (422), and 58° (511). Similarly, five intensity peaks also appeared in the spectrum of the pH 11 solution at 2θ 30.5° (220), 35° (311), 43° (440), 52.5° (422), and 57° (511). According to the literature, these intensity peaks are characteristic of Fe3O4 nanoparticles [10].

From the XRD result, the size of the produced nanoparticles can be measured. The average crystallite sizes of the nanoparticles in the pH 7, 9, and 11 solutions were determined to be 131, 29, and 15 nm, respectively. In terms of the size of the resulted nanoparticles, no similarity was found between the results of the XRD analysis and those of the TEM measurements, which is most likely due to the influence of the annealing process in the XRD preparation.

3.5. Magnetic test

A magnetic test was performed to confirm the results obtained, which indicated that Fe3O4 nanoparticles had been formed. Because magnetite (Fe3O4) exhibits magnetic properties, these should be found in the nanoparticles prepared in this work. Firstly, the starting materials were subjected to the
Figure 8. Magnetic test for the (a) extract, (b) precursor and (c) NaOH solutions.

Figure 9. Results of magnetic test after 1 h for biosynthesis in (a) pH=7, (b) pH=9 dan (c) pH=11

magnetic test, as seen in figure 8. After 1 h, it was clear that nothing was attracted to the magnet. The solutions of the biosynthetic reactions were then subjected to an external magnetic field. In the three samples, black lumps were magnetically attracted, gathering in the proximity of the external magnet. As a result, the three solutions became transparent after exposure to the external magnetic field for 1 h, as seen in figure 9.

4. Conclusions
The biosynthesis of FeO, magnetic nanoparticles using matoa leaf extract as reducing agent and Fe(NO3)3·9H2O as precursor was demonstrated. The size and shape of the nanoparticles, which experienced agglomeration, were not uniform, as extracted from the TEM analysis. The solution with pH 7 produced 10–20 nm nanoparticles, that with pH 9 produced 30–50 nm nanoparticles, and 5–10 nm nanoparticles were obtained from the solution with pH 11. The heterogeneous size of the nanoparticles was supported by PSA result, which indicated that the nanoparticles in the three samples were not similar in shape, but they were all large in size. The XRD result showed that the three samples were crystalline nanoparticles with Fe3O4 phase. Furthermore, it was found that the more basic the solution, the smaller the size and the better the crystallinity of the nanoparticles.

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