

Synthesis conditions and characterization of superparamagnetic iron oxide nanoparticles with oleic acid stabilizer

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ABSTRACT

Superparamagnetic iron oxide nanoparticles (SPIONs), part of magnetic nanoparticles, have been widely used in biomedical applications. Biocompatibility and magnetic properties make the SPIONs developed further by a lot of researchers. However, in the synthesis process, SPIONs can run into agglomeration. Oleic acid (OA) is one of the stabilizers to prevent agglomeration. This research aims to optimize the synthesis conditions and characterization of SPIONs with OA as a stabilizer. The synthesis of Superparamagnetic Iron Oxide Nanoparticles-Oleic Acid (SPIONs-OA) was performed using the coprecipitation method and was prepared with the addition of 0.75, 1.5, and 3%_{v/v} OA and stirring rate of 750, 1500, 3000, 6000, 9000, and 12,000 rpm. The characterization of hydrodynamic size and polydispersity index was evaluated by dynamic light scattering. Meanwhile, the crystal structure was observed by X-ray diffraction. Then, Fourier transform infrared spectroscopy (FTIR) was used to analyze structures. The results showed that the hydrodynamic size was dependent on OA concentrations and stirring rate. The addition of 1.5%_{v/v} OA and stirring conditions of 750 rpm resulted in the smallest hydrodynamic size and polydispersity index (83.71 ± 0.70 nm and 0.215 ± 0.01 nm, respectively). Based on the crystal structure analysis, the crystal shape was magnetic cubic, and the size of Fe₃O₄ crystallite changed from 11.38 to 5.61 nm. The FTIR indicated a strong chemical bond between the hydroxyl group of SPIONs and carboxylic acid of OA. In conclusion, the SPIONs-OA was successfully prepared with 1.5%_{v/v} OA concentrations and a stirring rate of 750 rpm.

Key words: Characterization, oleic acid, superparamagnetic iron oxide nanoparticles, synthesis conditions

INTRODUCTION

Currently, magnetic nanoparticles have been used on

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diagnostic therapies, tumor hyperthermia, targeted drugs, radioactive therapies, improved tissue repair, cell labeling, and cell separation.^[1,2] The magnetic nanoparticles have unique properties, mainly superparamagnetic iron oxide nanoparticles (SPIONs) due to superparamagnetic actions, chemical stability, magnetic properties, and good biocompatibility.^[3] Magnetic properties of SPIONs can be found in the core size below 20 nm which is often called "superparamagnetism."^[4]

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The particle size of SPIONs is obtained through a suitable synthesis method, one of which is the coprecipitation method which is widely chosen and considered in the synthesis of SPIONs because of its productivity and simplicity in the processing step. However, this method produces highly agglomerated SPIONs resulting in less effectiveness in biomedical application.^[5,6] Therefore, controlling the particle size and particle distributions of SPIONs is very crucial to avoid the SPION agglomerations and becomes a determinant of the synthesis process. SPIONs with particle sizes more than 100 nm can lose their magnetic properties easily because of oxidation and high chemical activity.^[7]

The addition of oleic acid (OA) as a stabilizer and surface effect in the synthesis of SPIONs is important in technological applications because it can control particle size and prevent aggregation between particles.^[8-10] Furthermore, OA is not only used as a stabilizer but also to prevent oxidation so that it can protect the layer of SPIONs.^[11] The previous research showed that optimization of stirring rate can affect the stabilization of SPIONs-OA.^[12] However, there is no report about the dual optimization of OA concentrations and stirring rate. This study, therefore, ascertains whether the optimization of OA concentrations and stirring rate can produce stable SPIONs-OA.

MATERIALS AND METHODS

Materials

$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, OA, and ammonium hydroxide 25% were purchased from Merck, Singapore.

Synthesis of superparamagnetic iron oxide nanoparticles-oleic acid

SPIONs were synthesized by using the coprecipitation method according to the previous literature with modification.^[8] The process was carried out in the environment of the nitrogen atmosphere and used a molar ratio of $\text{Fe}^{3+}:\text{Fe}^{2+}$ (2:1). The process started with dissolving $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (27.378 mM, 50 mL) and $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (13.413 mM, 50 mL) in demineralized water and heated to 60°C. Then, the process used vigorous stirring at 750–12,000 rpm. After the temperature reached 60°C, the ammonium hydroxide 25% was added at a speed of 1 mL in 6 s until the color of the solution turns black and the pH value of the solution is 11. After 30 min, OA with different concentrations (0.75%, 1.5%, and 3% v/v) was rapidly added to the reaction mixture and the temperature was raised to 80°C. After 1-h reaction, the suspension of SPIONs-OA was cooled and stored for further characterizations.

Characterization of superparamagnetic iron oxide nanoparticles-oleic acid

Hydrodynamic size and zeta potential analysis

Hydrodynamic size, polydispersity index, and zeta potential of SPIONs and SPIONs-OA were measured by

dynamic light scattering (DLS) using a Zetasizer Malvern Panalytical (Malvern Instruments, Malvern, UK) at room temperature.

Ultraviolet-visible spectroscopy analysis

The ultraviolet-visible (UV) spectroscopy spectra were recorded by JASCO V530. The spectra of SPIONs-OA and SPIONs were compared.

Morphology and structure analysis

The morphology and structure of SPIONs-OA were observed by transmission electron microscopy using Hitachi HT-7700, Institut Teknologi Bandung. The SPIONs-OA was placed on a 20-nm Cu: carbon transmission electron microscope (TEM) mesh grid.

Fourier transform infrared spectroscopy analysis

The chemical functional groups of SPIONs before and after OA coating were analyzed by Shimadzu Fourier transform infrared spectroscopy (FTIR) 840 OS. Samples were mixed with KBr before analyzing.

X-ray diffraction analysis

The crystallite size was calculated based on the X-ray diffraction (XRD) pattern by HighScore Plus software. The crystal structure of the SPIONs and SPIONs-OA was observed by XRD by using a PANalytical X'Pert.

RESULTS

Hydrodynamic size and zeta potential analysis

The DLS measurement result of SPIONs-OA is presented in Table 1 and Figure 1. The stirring rate and concentrations of OA affect the measurement result. The optimum result for hydrodynamic size, polydispersity index, and zeta potential is 83.71 ± 0.70 , 0.215 ± 0.01 , and -50.6 ± 0.61 , respectively, with the addition of 1.5% v/v OA and synthesis using 750 rpm (SPIONs-OA design B). The physical appearance of SPIONs and SPIONs-OA design B was observed by the suspension color change. After 2-h incubation at room

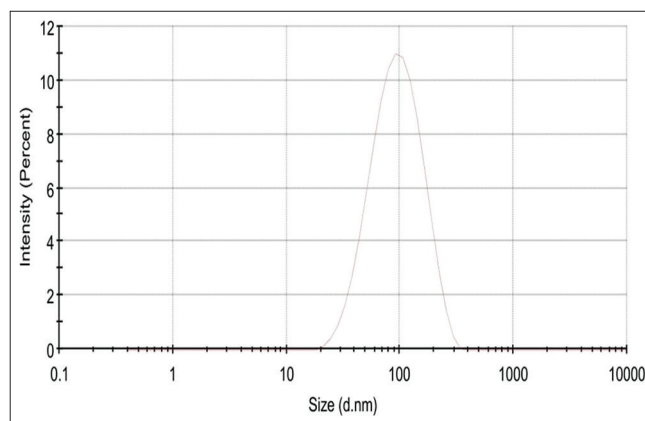


Figure 1: Particle size of superparamagnetic iron oxide nanoparticles-oleic acid design B

temperature, the SPION color changed from black to clear suspension. Meanwhile, the SPIONs-OA still formed the black suspension color, as shown in Table 2.

Ultraviolet-visible spectroscopy analysis

UV-visible spectroscopy analysis is shown in Figure 2. The wavelength of SPIONs showed spectral ranges between 330 and 450 nm, whereas OA represented maximum absorbance at 272.5 nm and 262 nm. Meanwhile, SPIONs-OA showed maximum absorbance at 273 nm and 373 nm.

Transmission electron microscopy analysis

The TEM analysis for SPIONs-OA is shown in Figure 3. The SPIONs-OA formed clusters of different sizes. However, each particle size was observed under 20 nm.

Fourier transform infrared spectroscopy analysis

The spectra of OA, SPIONs, and SPIONs-OA are shown

in Figure 4. For SPIONs-OA, the spectrum changed to lower frequencies at 3138 and 2935 cm^{-1} and showed Fe-O vibrations at 580 cm^{-1} whereas two bands of OA were shown at 2862 and 2928 cm^{-1} .

X-ray diffraction analysis

The XRD patterns of SPIONs and SPIONs-OA are shown in Figure 5. The position peaks of SPIONs-OA were similar to diffraction peaks of SPION samples. The XRD data were further analyzed using HighScore software. The result indicated that the crystal had a cubic shape.

DISCUSSION

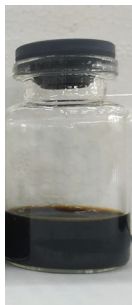




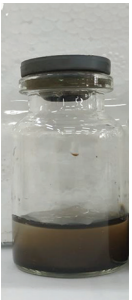


The synthesis of SPIONs-OA used the coprecipitation method with molar ratio $\text{Fe}^{3+}:\text{Fe}^{2+}$ (2:1). The solutions of Fe^{2+} and Fe^{3+} were mixed and added ammonia solution. The controlling of ammonium hydroxide titration showed

Table 1: Effect of oleic acid concentrations and stirring rate on dynamic light scattering results of superparamagnetic iron oxide nanoparticles with oleic acid stabilizer

Parameter	SPIONs-OA design results							
	A	B	C	D	E	F	G	H
Hydrodynamic size (nm)	86.37±0.97	83.71±0.70	211.67±7.04	116±0.61	92.3±1.13	142.37±13.27	117.8±3.65	172.63±3.29
Polydispersity index	0.256±0.03	0.215±0.01	0.609±0.06	0.395±0.02	0.215±0.03	0.240±0.006	0.279±0.03	0.296±0.04
Zeta potential (mv)	-42.6±2.08	-50.6±0.61	-88.13±4.92	-82±3.27	-38.03±1.17	-47.63±1.10	-48.07±6.15	-41.83±0.81
Stirring rate (rpm)	750	750	750	1500	3000	6000	9000	12000
Oleic acid concentrations (%)	0.75	1.5	3	0.75	0.75	0.75	0.75	0.75

SPIONs-OA: Superparamagnetic Iron Oxide Nanoparticles-Oleic Acid

Table 2: Physical appearance of superparamagnetic iron oxide nanoparticles and superparamagnetic iron oxide nanoparticles with oleic acid stabilizer design B

Description	After synthesis	After 15 (min)	After 30 (min)	After 2 (h)
SPIONs-OA design B				
SPIONs				

SPIONs-OA: Superparamagnetic Iron Oxide Nanoparticles-Oleic Acid, SPIONs: Superparamagnetic iron oxide nanoparticles

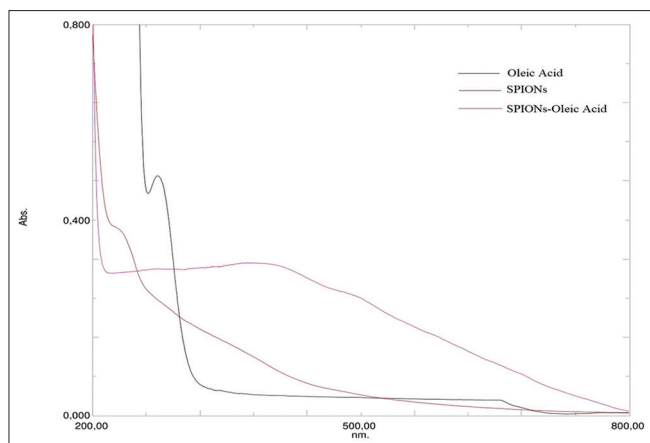


Figure 2: Ultraviolet-visible spectrum of superparamagnetic iron oxide nanoparticles, oleic acid, and superparamagnetic iron oxide nanoparticles-oleic acid

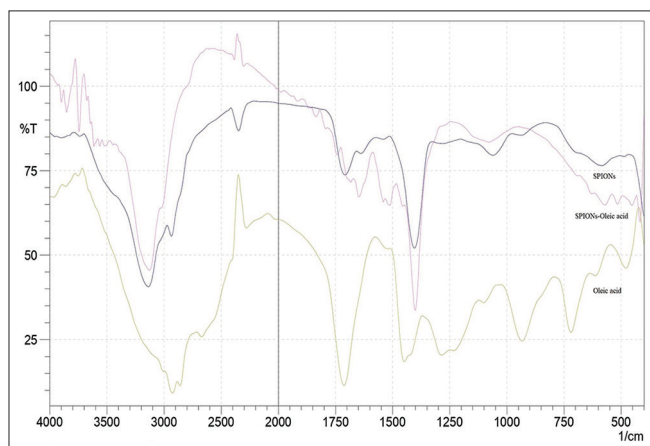


Figure 4: Fourier transform infrared spectroscopy spectra of superparamagnetic iron oxide nanoparticles, oleic acid, and superparamagnetic iron oxide nanoparticles-oleic acid

the narrow size distribution and changed the color of SPIONs-OA. The final color of the solutions was black. The processing of SPIONs-OA requires an oxygen-free environment to obtain the black precipitate of SPIONs.^[13] In the synthesis, the temperature must be maintained during the synthesis process because the temperature's reaction below 60°C will produce an amorphous hydrated oxyhydroxide and will be Fe₂O₃ formation. Controlling pH during the process is also important to control the particle size of SPIONs. The pH requirement of synthesis is 8 until 14 to obtain complete synthesis.^[14]

The hydrodynamic size of SPIONs-OA increased when the stirring rate was increased from 750 rpm to 1200 rpm because the high stirring rate will form agglomerations. The stirring rate from 750 to 9000 rpm increased hydrodynamic size from below 90 nm to hundreds of nanometers. In our case, the stirring rate with 12,000 rpm produces hydrodynamic size above 150 nm. Our study showed that the stirring rate affects the hydrodynamic size of

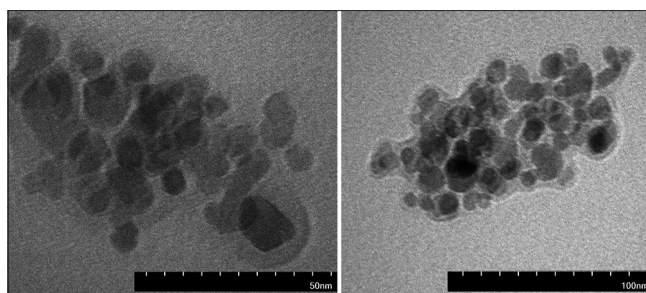


Figure 3: Image of superparamagnetic iron oxide nanoparticles-oleic acid from a transmission electron microscope

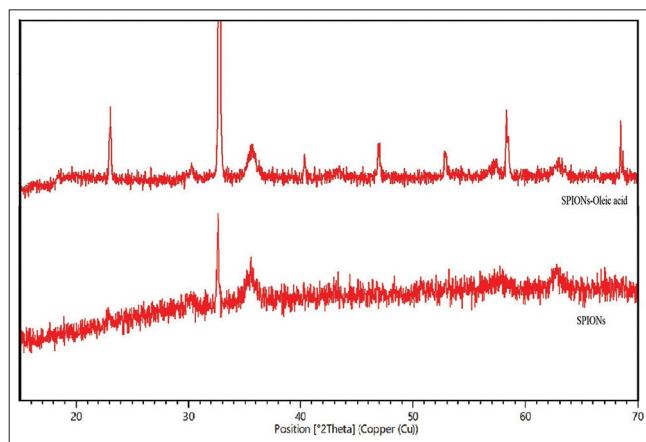


Figure 5: X-ray diffraction patterns of superparamagnetic iron oxide nanoparticles and superparamagnetic iron oxide nanoparticles-oleic acid

SPIONs and the successfulness of OA conjugation. The hydrodynamic size of pristine SPIONs showed 7492 nm before the addition of OA. The hydrodynamic size of the SPIONs decreased with the addition of OA concentration reaching 1.5%. However, the addition of OA more than 1.5% increased the hydrodynamic size. This result indicated the increasing of OA concentrations of more than 1.5% will produce agglomeration. The previous studies showed that the concentrations of OA influenced the hydrodynamic size of SPIONs.^[5] The addition of OA will form a protective monolayer that is linked to the surface of SPIONs and will form highly uniform and monodisperse SPIONs.^[15] The larger hydrodynamic size could be the reason for increasing the polydispersity index. The polydispersity indexes showed that the value <0.7, indicating the sample, has a narrow particle size distribution, which is often associated with particle stability in colloidal systems.^[16]

The zeta potential of pristine SPIONs was -25.3 mV, whereas SPIONs-OA ranged from -38.03 mV to -82 mV. These results were similar to the previous studies which stated that zeta potential changed after the addition of OA from -29.8 mV to -58.1 mV.^[17] The value of zeta potential is important in nanoformulations, and the high zeta potential (± 30 mV) can maintain a stable system. The visual result of TEM showed that the core of SPIONs-OA had particle sizes below 20 nm.

This result showed the differences between particle size from DLS because DLS gives the hydrodynamic diameter. The solvent elimination during the TEM process and preparation for analysis causes aggregation of SPIONs-OA and will form clusters.^[18] Nevertheless, the addition of OA will decrease the agglomeration because the OA coating on the surface of SPIONs will decrease contact among nanoparticles, thus restraining the growth of crystals, and the size will be smaller than pristine SPIONs.^[19]

The crystallite size of SPIONs after coating with OA changed from 11.38 nm to 5.16 nm because the addition of OA as a stabilizer can minimize the magnetic attraction between nano-sized particles.^[17] The magnetite crystal structure of SPIONs and SPIONs-OA was cubic structure and consisted of magnetite and maghemite. The diffraction peaks of SPIONs appear at angles of 30.05°, 32.47°, 35.44°, 43.12°, 57.10°, and 62.72° in the reflection plane 220, 221, 311, 400, 511, and 440, respectively. After coating with OA, the magnetite crystal structure was well-maintained and the characteristic peaks of SPIONs-OA appear at angles of 24°, 30.22°, 32.70°, 35.58°, 46.89°, 53.62°, 57.16°, and 68.46° corresponding to the diffraction peaks 210, 220, 200, 311, 220, 422, 511, and 400. The position peaks of SPIONs-OA were similar to diffraction peaks of SPION samples.^[12] The peaks indicated that the SPIONs-OA have a good crystal structure.

The FTIR showed two peaks of OA in the 2862 cm⁻¹ and 2928 cm⁻¹ regions, which are similar to the previous literature, which indicated symmetric and asymmetric CH₂ stretch.^[9,12] The peaks were also found in SPIONs-OA at 3138 cm⁻¹ and 2935 cm⁻¹, which indicated that OA molecules were absorbed into the SPION surface. The previous study reported that the C=O double bond strain was indicated by a peak at 1709 cm⁻¹.^[20] This was similar to pristine OA peaks at 1708 cm⁻¹ [Figure 4], indicating the carbonyl vibrational mode (C=O). Fe-O vibrations of pristine SPIONs were represented by peaks at 630 cm⁻¹ and 570 cm⁻¹. Meanwhile, the vibration was seen in SPIONs-OA at 580 cm⁻¹. Based on the FTIR spectra result, a strong chemical bond between the hydroxyl group of SPIONs and carboxylic acid of OA was developed. Thus, allowed the stabilization process of SPIONs with OA as a stabilizer.

CONCLUSION

The nonagglomerated SPIONs with OA have been successfully synthesized by a coprecipitation method. DLS analysis showed the hydrodynamic size was affected by concentrations of OA and stirring rate. The particle size below 20 nm was detected by TEM. XRD showed that the SPIONs-OA was similar to diffraction peaks of SPIONs and had smaller magnetic crystallite sizes compared to pristine SPIONs. The chemical bonds of SPIONs and OA were successfully formed.

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Conflicts of interest

There are no conflicts of interest.

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