Production of Iron Oxide Nanoparticles by Co-Precipitation method with Optimization Studies of Processing Temperature, pH and Stirring Rate

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Abstract. Iron Oxide Nanoparticle, maghemite ($\gamma$-Fe$_2$O$_3$) has received great interest and extensively used in biomedical field. Optimization studies were carried out in the production of $\gamma$-Fe$_2$O$_3$ nanoparticles by using co-precipitation method. Iron (II) chloride and iron (III) chloride were used as precursors which are dissolved in distilled water followed by centrifugation, drying and grinding process in order to obtain dried dark brown precipitated $\gamma$-Fe$_2$O$_3$ powder. The effect of different processing temperature (30 to 70°C), pH (10 to 12) and stirring rate (300 to 700rpm) towards crystallite size of $\gamma$-Fe$_2$O$_3$ were investigated by using Response Surface Methodology (RSM) and Central Composite Design (CCD). Based on analysis of variance (ANOVA), the determination coefficient, $R^2$ obtained was 0.9890 where stirring rate was the parameter that affected the most on the crystallite size. Optimization processing condition that produce smallest crystallite size of 7.3657 nm was 50 °C, pH 11.40 and 550 rpm by using Design of Expert software (DOE). Characterization of $\gamma$-Fe$_2$O$_3$ powder samples were evaluated by using different analytical tools such as Fourier Transform Infrared (FTIR), X-Ray Diffractometer (XRD) and Scanning Electron Microscope (SEM). Iron oxide group (Fe-O), hydroxyl (OH-) group and carbon dioxide (CO$_2$) were identified in FTIR spectrum. The characteristic peak occurring at 2θ = 35.4° indicated presence of $\gamma$-Fe$_2$O$_3$ in the samples. The $\gamma$-Fe$_2$O$_3$ particles appeared generally in spherical shape in SEM analysis.

1. Introduction

Recently, magnetic nanoparticles received a great interest and being extensively used around the globe. The superparamagnetic properties of iron oxide ($\gamma$-Fe$_2$O$_3$) nanoparticles have gained significant attention due to its attractive feature which contribute in nanomedical application. The concept of utilizing magnetic nanoparticles in gene and drug delivery was introduced in the late 1970s [1]. The first success of gene therapy with the used of $\gamma$-Fe$_2$O$_3$ nanoparticles was achieved in Italy in which modified gamma retroviral vector was utilized to transfer therapeutic gene to targeted stem cell in order to cure the life threatening disease [2]. The fatal disease such as cancer is treated with traditional approach such as surgery, immunotherapy, radiation therapy and chemotherapy. However, these approach have shortcoming of damaging normal cell [3]. Therefore, emergence of $\gamma$-Fe$_2$O$_3$ nanoparticles as non-viral vector have overcome the mentioned issue.

There are various type of magnetic nanoparticles available nowadays. Among them, iron oxide nanoparticles, IONPs maghemite ($\gamma$-Fe$_2$O$_3$) owns the unique properties such as nanoscale size, large ratio of surface-to-volume, low curie temperature and high coercivity [4]. Size and shape of IONPs significant influences the application in various field. $\gamma$-Fe$_2$O$_3$ nanoparticles has high surface activity with the size less than 100 nm are more efficient in biomedical application [5]. In spite of the progression of technology, it is still challenging to govern the size and shape of IONPs which will influence their magnetic properties [6].
In the last decades, different types of efficient method to synthesis iron oxide nanoparticles are developed by many researchers as to synthesis shape-controlled, monodispersed and biocompatible iron oxide nanoparticles. Numerous chemical, physical and biological route have been utilized in order to produce appropriate surface chemistry of magnetic nanoparticles [7]. The common chemical method including co-precipitation, sol-gel, hydrothermal, microemulsion and thermal decomposition. Among these methods, co-precipitation method is the most promising and cost effective method to produce γ-Fe2O3 nanoparticles due to its simplicity and high productivity. The characteristics of final product such as surface response, particle size and shape of the IONPs depends upon preparation technique. Only the nanosized γ-Fe2O3 nanoparticles with appropriate size, shape and purity has noteworthy impact in diverse biomedical applications and different scientific research areas.

In this research, co-precipitation method is applied to produce IONPs maghemite (γ-Fe2O3) for optimization and characterization studies with variable processing parameter such as processing temperature, pH and stirring rate. Therefore, γ-Fe2O3 nanoparticles can be produced by decisive control of these parameters. The γ-Fe2O3 nanoparticles formed from all the processing conditions will be systematically characterized and compared by using Scanning Electron Microscopy (SEM), X-Ray Diffraction (XRD) as well as Fourier Transform Infrared (FTIR).

2. Materials
2.1 Chemicals
Iron (II) chloride (FeCl2·4H2O, 99% pure), Iron (III) chloride (FeCl3, 98% pure, anhydrous), sodium hydroxide (NaOH, 98% pure) were purchased from Acros Organics and were used without further purification.

3. Procedures
3.1 Synthesis of maghemite nanoparticles
In this experiment, γ-Fe2O3 nanoparticles have been produced by co-precipitation method. 0.7954 g of iron (II) chloride, FeCl2·4H2O and 1.2974 g of iron (III) chloride, FeCl3 were each dissolved in distilled water. The mole ratio of iron (II) chloride and iron (III) chloride were fixed at 1:2 throughout the experiment. The solution was then mixed for 30 minutes by using hot plate with magnetic stirrer. In mixing steps, different processing temperatures, pH and stirring rates were regulated. After that, the mixed solution was separated by using centrifuge at 4000 rpm for 15 minutes. The separated precipitate was dried in the oven at temperature of 100 °C for 24 hours. Finally, the dried dark brown precipitated sample was collected and crushed into powder form by using a pestle and mortar. The chemical balanced equation for the reaction FeCl2·4H2O and FeCl3 is shown in Equation 1-3 where γ-Fe2O3 was the final product and water was the by-product:

\[ Fe^{2+} + 2Fe^{3+} + 80H^- \rightarrow Fe_3O_4 + 4H_2O \]  \hspace{1cm} (1)

\[ Fe_3O_4 + 0.25O_2 + 4.5H_2O \rightarrow 3Fe(OH)_3 \] \hspace{1cm} (2)

\[ 2Fe(OH)_3 \rightarrow γFe_2O_3 + 3H_2O \] \hspace{1cm} (3)

3.2 Particle Characterization Technique
γ-Fe2O3 nanoparticles formed from all the processing conditions can be systematically characterized in terms of functional groups, phase composition and crystallite size as well as morphology structure by using Fourier Transform Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD) and Scanning Electron Microscopy (SEM), respectively.

3.2.1 Fourier Transform Infrared Spectroscopy (FTIR)
Fourier Transform Infrared (FTIR) spectrometer (Perkin Elmer, Spectrum Version 10.02.00) was performed in order to investigate the functional groups in γ-Fe2O3 with the resolution of 4000-450 cm⁻¹. According to Nazari et al. [8], the most characteristic functional groups in the FTIR spectrum of synthesized maghemite nanoparticles
are the vibration of iron oxide group (Fe-O), hydroxyl group (OH-) and atmospheric carbon dioxide, CO2. The powder sample was grounded into fine powder before being placed onto the plate in order to obtained more accurate result.

3.2.2 X-ray Diffraction (XRD)

The phase composition of maghemite (γ-Fe2O3) was undertaken by using an X-Ray Diffractometer (XRD-6000 Shimadzu). The analysis of phase composition and crystallite size will be generated by using wavelength at λ=0.15406 nm over the 2θ range of 20° to 70° and proceed with scanning rate of 2°/min [9]. The peak broadening of XRD will be used to evaluate the crystallite size of IONPs based on the Scherrer formula.

3.2.3 Scanning Electron Microscopy (SEM)

SEM (JSM-6460LA) was used to examine the surface of γ-Fe2O3 nanopowder. A focused electron beam was scanned over a surface of sample to create an image when detection of secondary electrons. Cheng et al. [10] reported maghemite nanoparticle observed using SEM to be spherical shape.

3.3 Optimization study of γ-Fe2O3 nanoparticles by using Design-Expert® Software

DOE software (Design Expert 7.1.5) was applied to determine the number of experimental runs with the used of Response Surface Methodology (RSM) and Central Composite Design (CCD). The application of DOE able to obtain more information from fewer experiment. Response Surface Methodology (RSM) and Central Composite Design (CCD) were used to study the effect of parameters towards the response which is crystallite size of γ-Fe2O3. The parameters chosen were shown in Table 1.

| Factors | Name                | Units | Low (-α) | High (+α) |
|---------|---------------------|-------|----------|-----------|
| A       | Processing temperature | °C    | 30       | 70        |
| B       | pH                  |       | 10       | 12        |
| C       | Stirring rate       | rpm   | 300      | 700       |

4. Result and Discussion

4.1 Maghemite nanoparticles characterization analysis

4.1.1 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectrum analysis is an important tool to investigate the biomolecules present in IONPs. The spectra generated by FTIR was investigated to identify the functional groups and organic compounds contained in nanoparticles. The most characteristic functional groups in the FTIR spectrum of synthesized maghemite nanoparticles, γ-Fe2O3 are vibration of iron oxide group (Fe-O), hydroxyl group and atmospheric carbon dioxide [8]. Five samples of dark brown powder form of γ-Fe2O3 nanoparticles with different processing temperature, pH and stirring rate is selected and investigated by using Perkin Elmer Spectrum Version FTIR equipment with the resolution of 4000-450 cm-1. The tested ATR-FTIR spectrum of γ-Fe2O3 nanoparticles result are shown in the Figure 1.

The tested sample powder of IONPs, maghemite (γ-Fe2O3) shows infrared absorption spectra as in Figure 1. The result obtained for five samples shows the usual band of IR absorption. The absorption band assigned to the stretching mode of hydroxyl group of water molecules can be observed at around 3391-3403 cm-1. Alternatively, for O-H stretch vibration of hydrogen-bonded hydroxyl group, the adsorbed water band obtained was in the range of 1607-1624 cm-1. The stretching mode of hydroxyl O-H group occurred at around 3100-3600 cm-1 was the major evidence of presence of OH group of water [11]. Two absorption bands appeared at 3,170 and 3,408 cm-1 represented the stretching band of O-H group [8]. Chakrabarti et al. [12] reported that γ-Fe2O3 nanoparticle at 1621 cm-1 and 3387 cm-1 absorption peak corresponded to the adsorbed water. Presence of hydroxyl group indicated crystallization of water in the powder. Hence, the result of the absorption band for O-H group was successfully achieved.

Chauhan et al. [13] stated presence of iron oxide functional group occurred at region of low frequency. Octahedral and tetrahedral sites of maghemite occurred vibration of Fe-O at absorption bands of 630 cm-1 to
550 cm\(^{-1}\) range [14]. From the Figure 1, the strong and obvious absorption peak that obtained was in the range of 631-634 cm\(^{-1}\) indicated the vibration of Fe-O functional group of maghemite nanoparticles. As the maghemite molecule contained main elements of iron and oxygen, existence of Fe-O group is necessary. Absorption peak for Fe-O group can be observed for the five tested sample powder. According to Aliahmad and Nasiri Moghaddam [15], the intense peak for Fe-O group at 586 cm\(^{-1}\). On the other hand, the absorption peaks at 636 cm\(^{-1}\) and 636 cm\(^{-1}\) assigned to Fe-O group of maghemite [16].

According to Aliahmad and Nasiri Moghaddam [15], atmospheric carbon dioxide can be observed at 2361 cm\(^{-1}\). From Figure 1, IR absorption band at 2922 cm\(^{-1}\) shows the presence of carbon dioxide as maghemite nanoparticle exposed to atmosphere that contained carbon dioxide. Besides, 2337 cm\(^{-1}\) and 2361 cm\(^{-1}\) wavelength in absorption spectrum also indicated existence of atmospheric carbon dioxide [12]. Formation of IR band occurred only in the presence of dipole moment changing in the molecules. In short, dipole moment changes during the molecular vibration [17].

Nazari et al. [8] stated that pure maghemite contained other peaks such as at 892 cm\(^{-1}\) and 795 cm\(^{-1}\). The result of the five samples presented the remaining peaks of maghemite in the range of 804-898 cm\(^{-1}\). Furthermore, the absorption peak at 1330-1337 cm\(^{-1}\) can be considered as vibration of ferrophase complex. However, there is one of the absorption spectrum which is overlap with the others sample, probably due to the polarization effect that affects the light reflection. In addition, crystalline sample considered a nuisance in ATR-FTIR analysis as will reflect the beam and lead to wrong spectra. Hence, crushing of crystalline sample into fine powder is necessary due to the sensitivity of the instrument.

FTIR result obtained clearly indicated three main functional groups, Fe-O, OH- and CO2 were observed in their respective IR region. The formation of spectra for all the tested IONPs powder were followed the same trend regardless the effect of processing factors such as pH, temperature and stirring rate. Hence, the IONPs tested are free from impurities.

![Figure 1. ATR-FTIR spectrum of maghemite.](image)

4.1.2 X-ray Diffraction (XRD)
The phase composition of IONPs maghemite (γ-Fe2O3) was undertaken by using an X-Ray Diffractometer (XRD-6000 Shimadzu). Determination of crystallite size and phase composition was generated by using wavelength of Cu-K\(\alpha\) radiation (\(\lambda = 0.15406\) nm) over 2\(\theta\) range of 20 to 70\(^{\circ}\) proceed with scanning rate of 2\(^{\circ}\)/min. Figure 2 shows the diffraction pattern of maghemite (S4) that synthesized at a processing temperature of 50\(^{\circ}\)C, pH 11 and stirring rate of 500 rpm. There are three different diffraction peaks confirming to the crystal planes of crystalline IONPs [18]. Moreover, diffraction pattern correspond to the formation of maghemite has been confirmed by indicating the characterization peak occurring at 2\(\theta\) = 35.4\(^{\circ}\) with (311) plane appeared in all of the tested samples. Database International Centre for Diffraction Data (ICDD) was applied during the analysis
and the characteristic of maghemite was verified. The X-ray diffractogram of all the tested samples that synthesized from different processing condition were almost similar.

![Figure 2. XRD pattern of maghemite, $\gamma$-Fe$_2$O$_3$ at different processing condition.](image)

By using Scherrer equation 4, crystallite size of synthesized IONPs was calculated with the use of full width at half maximum at the peak broadening of diffraction reflection which occurred at $2\theta = 35.4^\circ$. The result of crystallite size was tabulated in Table 2.

$$X_s = \frac{0.94 \lambda}{\text{FWHM} \cos \theta}$$

(4)

Where Scherrer constant 0.94, $X_s$ is the crystallite size (nm), FWHM is full width at half maximum of diffraction peak (rad), $\lambda$ is the wavelength radiation for Cu-Kα and $\theta$ is diffraction angle ($^\circ$).

### 4.1.3 Scanning Electron Microscopy (SEM)

![Figure 3. SEM image of IONPs at different stirring rate with constant temperature and pH.](image)
The morphology study of IONPs maghemite was studied by using JSM-6460LA scanning electron microscope (SEM). Figure 3 depicted the SEM pictures of IONPs maghemite at the same processing temperature (50 °C) and pH 11 with different stirring rates (300 rpm and 700 rpm). The particles were generally spherical shape with some is cuboid-like formed at a lower stirring rate while the particles with spherical shape with some elongated ellipse-like were observed at higher stirring rate. The agglomerated nanoparticles may be due to magnetic characteristic of IONPs [19]. The agglomeration of IONPs also indicated the small surface to volume ratio of the particles. Moreover, the non-spherical shape formation can be explained by the shear produced during stirring process [20]. High stirring rate possess induced shear in the mixing zone that contribute to the vortex within the system which built up aggressive flow behavior and caused some of the formation of elongated particles. In addition, there is high energy dissipation at high stirring rate which enhance the nucleation rate, crystal growth mechanism and subsequent morphology is affected [21]. Therefore, IONPs showed a high tendency to agglomerate where they tended to stick together. Based on Table 2, it can be seen that 27.6080 nm and 9.9870 nm of IONPs were formed at 300 rpm and 700 rpm respectively with the constant processing temperature of 50 °C and pH 11.

4.2 Optimization study of maghemite nanoparticles by using Design-Expert® Software

In this model design, Design Of Experiment software (Design Expert 7.1.5) was applied to determine the amount of experimental runs to optimize the synthesis of IONPs with the used of Response Surface Methodology (RSM). Central Composite Design (CCD) is an experimental model design which is useful to study the effect of processing temperature, pH and stirring rate towards the crystallite size of IONPs. There are three parameters were set at low (-α) and high (+α) level while the response set as crystallite size in quadratic model. This model consists of 20 experimental runs with 6 central points of the parameters in order to determine the crystalline size of IONPs under different combination of conditions. Table 2 shows the outcome of crystallite size of IONPs which is a responses that calculated by using Scherrer’s formula based on the full width half maximum of X-ray Diffraction (XRD) spectrum diffraction peak.

Table 2. Crystallite size (nm) of IONPs for different process conditions.

| Run | Factor 1 | Factor 2 | Factor 3 | Response: Crystallite size of IONPs (nm) |
|-----|----------|----------|----------|------------------------------------------|
| A: Temperature (°C) | B: pH | C: Stirring rate (rpm) |
| 1  | 50.00    | 11.00    | 500.00   | 9.2472                                   |
| 2  | 50.00    | 11.00    | 500.00   | 9.5321                                   |
| 3  | 70.00    | 11.00    | 500.00   | 26.2270                                  |
| 4  | 50.00    | 11.00    | 500.00   | 9.0642                                   |
| 5  | 38.00    | 11.60    | 380.00   | 25.5622                                  |
| 6  | 62.00    | 11.60    | 620.00   | 11.0010                                  |
| 7  | 50.00    | 12.00    | 500.00   | 12.6532                                  |
| 8  | 38.00    | 11.00    | 620.00   | 25.1235                                  |
| 9  | 50.00    | 11.00    | 500.00   | 9.5121                                   |
| 10 | 50.00    | 11.00    | 700.00   | 9.9870                                   |
| 11 | 50.00    | 11.00    | 500.00   | 9.6421                                   |
| 12 | 62.00    | 11.60    | 380.00   | 25.7895                                  |
| 13 | 38.00    | 10.40    | 380.00   | 25.0312                                  |
| 14 | 50.00    | 11.00    | 500.00   | 11.1102                                  |
| 15 | 62.00    | 10.40    | 380.00   | 22.0933                                  |
| 16 | 62.00    | 10.40    | 620.00   | 18.1211                                  |
| 17 | 38.00    | 11.60    | 620.00   | 14.3334                                  |
| 18 | 30.00    | 11.00    | 500.00   | 31.5491                                  |
| 19 | 50.00    | 10.00    | 500.00   | 18.4072                                  |
| 20 | 50.00    | 11.00    | 300.00   | 27.6080                                  |
4.2.1 Analysis of Anova

Analysis of variance (ANOVA) can be used to determine the reliability of a design model by reducing random viability [22]. ANOVA is a structural system design by taking account on the presence of uncertainties involved in the reliability of the structure of a model. Structural reliability is a theoretical basis for determining the likelihood of failure, which is also known as reliability index of structural systems. The ANOVA test result was accomplished in order to validate the quadratic polynomial model’s significance. Table 3 below tabulated the ANOVA for optimization study of quadratic model whereas R-Squared value for response parameter is shown in the Table 4.

**Table 3. Analysis of variance (ANOVA) for optimization study quadratic model.**

| Source         | Sum of squares | Df | Mean Square | F value | p-value |
|----------------|----------------|----|-------------|---------|---------|
| Model          | 1135.13        | 9  | 126.13      | 99.69   | <0.0001 significant |
| A: Temperature | 35.43          | 1  | 35.43       | 28.00   | 0.0004  |
| B: pH          | 39.96          | 1  | 39.96       | 31.58   | 0.0002  |
| C: Stirring rate | 259.51       | 1  | 259.51      | 205.11  | <0.0001 |
| AB             | 5.84           | 1  | 5.84        | 4.62    | 0.057   |
| AC             | 7.27           | 1  | 7.27        | 5.74    | 0.0375  |
| BC             | 61.26          | 1  | 61.26       | 48.42   | <0.0001 |
| A²             | 628.43         | 1  | 628.43      | 496.69  | <0.0001 |
| B²             | 50.98          | 1  | 50.98       | 40.29   | <0.0001 |
| C²             | 132.83         | 1  | 132.83      | 104.99  | <0.0001 |
| Residual       | 12.65          | 10 | 1.27        |         |         |
| Lack of fit    | 9.99           | 5  | 2.00        | 3.75    | 0.0866  |
| Pure error     | 2.66           | 5  | 0.53        |         |         |
| Cor total      | 1147.78        | 19 |             |         |         |

*df – degree of freedom

**Table 4. R-Squared value for response parameter.**

| Parameters            | Value |
|-----------------------|-------|
| Standard deviation    | 1.12  |
| R-Squared             | 0.9890|
| Adjusted R-Squared    | 0.9791|
| Predicted R-Squared   | 0.9306|
| Mean                  | 17.58 |
| C.V. %                | 6.40  |
| PRESS                 | 79.64 |
| Adequate precision    | 26.889|

Based on the Table 3, the F-value of model obtained is 99.69 whereas p-value of the model is below 0.0001. This implies that the individual terms in the response model generated was statistically significant on interaction effect and only 0.01% chance of noise occurrence. The p-value expressed the significance of each factor, if the factor has a p-value below 0.05 indicate that the model terms are significant with a confidence level of 0.95 [23].

In this ANOVA analysis, the variable A, B, C, AC, BC, A², B², and C² had significant effect on the response which is crystallite size of IONPs due to the p-value less than 0.05. However, the variables AB showed insignificant effect towards response due to the p-value 0.057 is greater than 0.05. Those insignificant variables were retained because there is no influence of model reduction on insignificant variables. “Lack of fit F-value” have to be insignificant so that the model fit well in the experimental design. In this analysis, 3.75 lack of fit was obtained and there is 8.66% chance that F-value of Lack of Fit this large might occur due to the noise. The
pure error implied the variability of the observations of each treatment. In order to reduce large variance of experimental data, more replicates of experiments shall be conducted since 3 replication of experiments generated by DOE have been carried out.

In order to assess the model performance, analysis of determinates coefficients R-squared ($R^2$), predicted R-squared, adjusted R-squared and adequate precision were tabulated in Table 4. Based on Table 4, the $R^2$ obtained was 0.9890 which close to value of 1.0 indicated the model was highly reliable and fit to actual data. Predicted $R^2$ is a measurement of predictive capability of the regression model. The difference between “predicted $R^2$” (0.9306) and “adjusted $R^2$” (0.9791) is 0.05. This indicated that the “predicted $R^2$” is in a reasonable agreement with “adjusted $R^2$”. If there is difference greater than 0.2 will cause data problem or model distribution. “Adequate precision” compares the range of the values predicted at design points with the prediction of average error. In other words, it is a measure of the experimental signal to noise ratio. From Table 4, “adequate precision” of 26.889 greater than 4 indicated that the performance of model is in an adequate signal situation to circumnavigate the space of design [24].

The coefficient of variation (C.V.) is a measure of relative variability on the ratio of standard deviation to average mean. The higher the C.V 6.4%, the higher the dispersion level around the mean and less reliable of this optimization study.

4.2.2 Analysis of regression

Table 5 shows coded parameters for crystallite size of IONPs, maghemite ($\gamma$-Fe$_2$O$_3$). The final equation of model in terms of coded parameters was developed by using the experimental data that listed as below.

| Factor        | Coefficient estimate |
|---------------|----------------------|
| Intercept     | 9.71                 |
| A: Temperature| -1.61                |
| B: pH         | -1.71                |
| C: Stirring rate | -4.36               |
| AB            | 0.85                 |
| AC            | -0.95                |
| BC            | -2.77                |
| $A^2$         | 6.60                 |
| $B^2$         | 1.88                 |
| $C^2$         | 3.04                 |

The relationship between the factors and response has been developed by using Central Composite Design (CCD). A mathematical regressive model in terms of coded parameters which was linearly correlated related to variables that influenced the crystallite size of IONPs is shown in Equation 5.

\[
\text{Crystallite size} = 9.71 - 1.61 \cdot A - 1.71 \cdot B - 4.36 \cdot C + 0.85 \cdot AB - 0.95 \cdot AC - 2.77 \cdot BC + 6.60 \cdot A^2 + 1.88 \cdot B^2 + 3.04 \cdot C^2
\]  

Internally studentized residual is the residual (difference between actual value and predicted value) divided by that residual estimated standard deviation. Based on Figure 4, normal probability plot of internally studentized residuals was scattered closely along the positive slope. This showed an accurate model with good agreement throughout the operating variable ranges as the residuals distributed in a linear line. Besides, Figure 5 shows there was a great correspondence between the actual value (experimental data) and predicted value (evaluated by DOE). In short, the result from both graph considered as a good fit to the quadratic model.
4.2.3 Analysis of model

Response Surface Methodology (RSM) is a powerful optimization tool used to determine the response of that crystallite size of IONPs, maghemite (γ-Fe2O3) at optimum condition for each parameters. Three parameters were employed in this study such as pH, temperature and stirring rate. Figure 6, 8 and 10 showed an egg-shaped 2D contour plot which indicated the relationship between the related study parameters. Furthermore, Figure 7, 9 and 11 illustrate 3D surface of hyperbola minimum hill with a minimum point located at the bottom of experimental design. The minimum point represented the minimum value of particle size. All the factors exerted positive effect towards the minimization of crystallite size as the result exhibit downward quadratic curve line.

4.2.3.1 Effect of processing temperature and pH towards IONPs crystallite size

The optimum region for processing temperature (A) was in the range of 44.3 °C to 58 °C whereas for pH (B) was in the range of 10.60 to 11.60. The particle size of γ-Fe2O3 decreased when the pH increased when temperature is fixed at 50 °C. For all the pH value, it shows that particle size decreased when processing temperature increased from 38 °C to 50 °C. However, the particle size started to increase when the processing temperature above 50 °C. The increased particle size at higher temperature can be explained by the Ostwald-ripening phenomenon where normal mechanism of growth is distorted and particle is redeposited onto the stable nuclei [9]. Besides, gradual decreased in size of particle with increased temperature due to the accelerated nuclei
collision rate in which extent of aggregation within the particle is reduced by the large energy [25]. Yusoff et al. [9] agreed with this result where the particle size decreased when temperature and pH increased.

4.2.3.2 Effect of processing temperature and stirring rate towards IONPs crystallite size
Based Figure 8 and Figure 9 below, the optimum region for processing temperature (A) was 44.30 °C to 60.30 °C while for stirring rate (C) was 470 rpm to 619 rpm. At temperature of 50 °C, the particle size decreased when the stirring rate increased. This is due to high degree of agitation, the contribution of high energy to the suspension medium resulted dispersion of solution into smaller precipitate. At 500 rpm, the particle size of $\gamma$-Fe2O3 decreasing from 38 °C to 50 °C but particle size started to increase when the temperature above 50 °C. Larger size of particles are formed at low stirring velocity because the uniformity of the reaction solution are distorted [9]. The nucleating species tends to aggregate when it is not equally dispersed throughout the solution [26]. In contrast, high stirring rate resulted smaller particle size as the mixture contain greater seed formation which led to smaller particle size. Therefore, stirring rate will affect nucleation and aggregation which in turn affect the size of particles [27].
4.2.3.3 Effect of pH and stirring rate towards IONPs crystallite size

Figure 10 and 11 clearly showed that in the range of pH 10.80 to pH 11.60 and 490 rpm to 619 rpm was the optimum region for the parameter pH (B) and stirring rate (C) respectively. Besides, particle size decreased when the stirring rate increased. This is due to the homogeneity of reaction solution is enhanced at high mechanical stirring. On the other hand, particle size decreased when pH increased from 10.40 to 11 but particle size started to increase after pH 11. There is excess ion present in the growth phase when pH of solution is increased which lead to low interfacial tension. Hence, the smaller particle size is formed at this state as the reaction rate is faster and more initial nuclei capable to precipitate. It is also observed that smaller particle size can be obtained at higher stirring rate as in agreement in the study of Yusoff et al. [9].

![Figure 10](image1.png) 2D contour plot on the interaction of B (pH) and C (stirring rate) towards particle size.  
![Figure 11](image2.png) 3D surface plot on the interaction of B (pH) and C (stirring rate) towards particle size.

4.2.3.4 Validation of experimental model

Table 6 demonstrate the desired goals for the factors and response of particle size γ-Fe2O3 whereas Table 7 show the validation test result for optimization verification. Based on Table 7, the selected solution generated by Design Expert Software represented the optimum condition to produce the smallest particle size of γ-Fe2O3. The smallest particle size obtained was 7.3657 nm at 50 °C, pH 11.40 and 550 rpm. The difference between the value of predicted size (7.4965 nm) and actual experimental value was only small percentage error of 1.75 %. Since the percentage error was less than 5 %, it can be concluded that selected solution condition has been validated.

| Table 6. Desired goals for factors and response of particle size γ-Fe2O3. |
| --- |
| **Factor** | **Goal** | **Lower limit** | **Upper limit** |
| Processing temperature | In range | 30 °C | 70 °C |
| pH | In range | 10 | 12 |
| Stirring rate | In range | 300 rpm | 700 rpm |
| Particle size | minimize | 9.0642 nm | 31.5491 nm |

| Table 7. Validation test result for optimization verification. |
| --- |
| **Run** | **A:** Processing temperature | **B:** pH | **C:** Stirring Rate | **Current response of IONPs crystallite size (nm)** | **Predicted** | **Experimental** | **Error (%)** |
| 1 | 50 | 11.40 | 550.00 | 7.4965 | 7.3003 | 2.62 |
| 2 | 50 | 11.40 | 550.00 | 7.4965 | 7.4941 | 0.03 |
| 3 | 50 | 11.40 | 550.00 | 7.4965 | 7.3027 | 2.59 |
| Average | 50 | 11.40 | 550.00 | 7.3657 | 1.75 |
5. Conclusion

In conclusion, the overall goal of this project has been achieved since the characterization and optimization studies on the synthesis of IONPs, maghemite (γ-Fe2O3) by using co-precipitation has been successfully carried out. The investigation effect of processing parameters towards the smallest particle size of γ-Fe2O3 has been synthesized at higher temperature, higher pH and higher stirring rate. The method that has been utilized is co-precipitation owing to high convenience, high production and low cost. The statistical study was performed by Response Surface Methodology (RSM) with the use of CCD for the optimization of γ-Fe2O3 production. The determination coefficient (R2) obtained was 0.9890. The model F-value obtained was 99.69 and p-value < 0.0001 indicated this model was statistically significant. The optimization of γ-Fe2O3 production was completed at 50 °C, pH 11.40 and 550 rpm and 7.3657 nm of γ-Fe2O3 produced as the smallest crystallite size. The result of Fourier Transform Infrared Spectroscopy (FTIR) analysis showed the production of γ-Fe2O3 by using co-precipitation revealed three main functional group, OH-, CO2 and Fe-O group. X-ray Diffraction (XRD) analysis has confirmed the presence of γ-Fe2O3 occurred at the peak of 2θ = 35.4°. Besides, Scanning Electron Microscopy (SEM) analysis showed agglomerated nanoparticles at lower stirring rate while spherical shaped were obtained at higher stirring rate.

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