CHARACTERISTICS AND MAGNETIC PROPERTIES OF CHITOSAN-COATED Fe₃O₄ NANOPARTICLES PREPARED BY EX-SITU CO-PRECIPITATION METHOD

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ABSTRACT

Chitosan-coated iron oxide nanoparticles were synthesized by ex-situ co-precipitation method. Ammonia solution was dropped into the solution containing ferrous and ferric salts through a syringe pump to form Fe₃O₄ nanoparticles. Nanoparticles produced were then coated and crosslinked with chitosan and tripolyphosphate/sulfate, respectively. The physical and magnetic properties of chitosan-Fe₃O₄ nanoparticles were characterized using Scanning Electron Microscopy (SEM), X-Ray Diffraction (XRD), and Electron Spin Resonance (ESR) respectively. The results showed that the ratio enhancement between chitosan:Fe₃O₄ increased the particle size, while the crystallite size decreased. Morphology and particle size were also influenced by the ratio of crosslinkers. It was found that the higher tripolyphosphate content contributed to the smaller size and more spherical morphology. In addition, the influence of crosslinking time toward crystallite size was determined by tuning stirring time. The result showed that the longer duration of crosslinking time, the larger crystallite size of chitosan-coated Fe₃O₄ was obtained.

Keywords: Nanoparticles, Iron oxide, Co-precipitation, Chitosan, Crosslinker.

INTRODUCTION

Drug delivery system plays an important role to reduce adverse effects of excessive drug supply for human as well as to conduct drug distribution and lead it into the targeted cell.¹,² Materials for drug delivery applications can be produced from nanoparticles. Nanoparticles have promising potential for use in various applications due to their unusual characteristics compared to their bulk materials. Magnetic nanoparticles are a type of inorganic nanoparticles that widely developed in various studies and application fields because of its magnetic properties. As reported by Wu and co-workers, both iron oxide magnetite (Fe₃O₄) and maghemite (γ-Fe₂O₃) have biocompatibility to the human body.³

Chitosan is a polysaccharide that has biocompatibility and biodegradability characteristics. It can be obtained from the product of deacetylated chitin. Chitosan has better stability in a neutral condition. However, it can be dissolved in acid condition under pH 6.5.⁴ Chitosan can be used along with magnetic (Fe₃O₄) nanoparticles in drug delivery system. Surface modification of Fe₃O₄ by chitosan coating is needed to stabilize Fe₃O₄ nanoparticles, as well as avoid oxidation processes. Furthermore, it is also intended to provide a functional group on the surface of nanoparticles that can be bound to other molecules, such as either proteins or medicines (drugs).³

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Chitosan nanoparticles can be manufactured by using several methods, and one of them is ionic gelation method. In this approach, chitosan nanoparticles are obtained through additional of crosslinking agent. Crosslinking agent had also contributed to maintaining the stability of chitosan nanoparticles in acid condition. For a biomedical application, it plays a major role in drug release control. Tripolyphosphate (TPP) is a non-toxic anionic crosslinker that can interact with chitosan to form a crosslinking network through ionic gelation process. TPP could also be combined with other anionic crosslinking agents such as sulfate and citrate. Shu and Zhu explained that combination of either TPP/sulfate or TPP/citrate as co-crosslinking agents exhibited better spherical morphology of the materials. Therefore, in this study, chitosan-Fe$_3$O$_4$ nanoparticles were synthesized by using the combination of crosslinking agents, TPP and sulfate. Some parameters such as chitosan:Fe$_3$O$_4$ ratio, the combination of crosslinkers ratio, as well as crosslinking time were examined to observe physical characteristics and magnetic properties of the nanoparticles products.

**EXPERIMENTAL**

**Material**

The low molecular weight of chitosan (75-85% deacetylation degree) and sodium tripolyphosphate (Na$_5$P$_3$O$_10$, 85% (w/w)) were purchased from Sigma-Aldrich (Singapore). Other chemicals, such as ferric chloride (FeCl$_3$·6H$_2$O, 99% (w/w)), ferrous chloride (FeCl$_2$·4H$_2$O, 98% (w/w)), ammonia (NH$_3$, 25% (v/v) in H$_2$O), and sodium sulfate (Na$_2$SO$_4$, ≥ 99% (w/w)), acetic acid glacial (CH$_3$COOH, 100% (v/v)) were purchased from Merck (Indonesia).

**Synthesis of Magnetic Nanoparticles**

Magnetic nanoparticles of Fe$_3$O$_4$ were synthesized using co-precipitation method by mixing of FeCl$_2$·4H$_2$O (1.72 g) and FeCl$_3$·6H$_2$O (4.70 g) salts in 50 mL de-ionized water. Ammonia solution (3 mol L$^{-1}$) was added dropwise into the salt mixture through syringe pump (10 mL h$^{-1}$) until the solution reached pH 10, and the black precipitate was obtained as illustrated in Fig.-1. During this process, the mixture solution was stirred by a magnetic stirrer. The process was finished by washing the black precipitate of Fe$_3$O$_4$ with de-ionized water to remove any alkali metals, and separated by magnetic decantation. Afterward, it was suspended in 125 mL of de-ionized water. Before being characterized, the black precipitates of Fe$_3$O$_4$ in de-ionized water were dried by freeze drying process.

**Preparation of Chitosan-Coated Magnetic Nanoparticles**

Chitosan solution was prepared by dissolving 0.5 g of chitosan in 100 mL of 1% (v/v) acetic acid to form 0.5% w/v chitosan solution. A 10 mL of Fe$_3$O$_4$ in aqueous solution (correspond to 0.15 g of dried Fe$_3$O$_4$) was then dropped into chitosan solution with various volume (10, 20, and 30 mL) with constant stirring for 30 minutes to obtain ferrogel solution for examining the effect of chitosan and Fe$_3$O$_4$ ratio. The
combination of TPP and sulfate as crosslinkers with total volume of 10 mL (ratio TPP:sulfate 3:1) were added dropwise into ferrogel solution using a syringe pump with constant stirring for 1 hour at ambient temperature. Then, the mixture was further stirred for 1 hour. The mixtures were washed with de-ionized water, separated by magnetic decantation, and suspended in 50 mL of de-ionized water. Before characterization process, the chitosan-Fe$_3$O$_4$ nanoparticles in deionized water were dried by freeze drying. Furthermore, to understand the effect of crosslinkers ratio and crosslinking time, the nanoparticles were produced according to the procedures above. However, the preparation was conducted with various TPP:sulfate volume ratio (1:1, 3:1, and 5:1) and 1-hour crosslinking time at constant chitosan:Fe$_3$O$_4$ ratio 1:1. In addition, to investigate the effect of crosslinking time, the mixtures were further stirred for 1, 2, and 3 hours at constant chitosan:Fe$_3$O$_4$ ratio (1:1) and TPP:sulfate ratio (3:1).

Characterization

X-ray characterization of the synthesized products was performed by X-ray diffractometer (Philips type X’pert MPD; from PANalytical B.V., Eindhoven, Netherlands) at room temperature using Cu K$_\alpha$ radiation ($\lambda$ =1.5406 Å) with the 2\(\theta\) range of 15 – 65\(^\circ\). Crystallite size of the chitosan-Fe$_3$O$_4$ nanoparticles was evaluated from X-ray analysis data using modified Debye-Scherrer Equation as given in Eqn.1\(^{15}\) as:

\[
\ln \beta = \ln \frac{K \lambda}{L \cos \theta} = \ln \frac{K \lambda}{L} + \ln \frac{1}{\cos \theta}
\]

where \(\beta\) is full-width at half-maximum diffraction peak (FWHM), \(K\) is Scherrer constant related to crystallite shape, \(\lambda\) is x-ray wavelength (nm), \(\cos \theta\) is Bragg angle (rad), and \(L\) is crystallite size (nm). Then, the intercept of ln \(K/L\) as well as the crystallite size was obtained by plotting ln \(\beta\) against ln \((1/\cos \theta)\).\(^{15}\) Homogeneity of formed crystallite size was evaluated using a statistical method of Gaussian distribution as displayed in Eqn. 2 as:

\[
(L) = \frac{1}{L \sigma \sqrt{2\pi}} \exp \left( -\frac{1}{2} \frac{(L-L_0)^2}{\sigma^2} \right)
\]

Where \(L\) is observed crystallite size, \(\sigma\) is the standard deviation, and \(L_0\) is average crystallite size of the nanoparticles.

The XRD data were also used to determine iron content by Materials Analysis using Diffraction (MAUD) software. The size, morphology, and elemental analysis of the synthesized product of Fe$_3$O$_4$ and Chitosan-Fe$_3$O$_4$ nanoparticles were determined by Scanning Electron Microscopy-Energy Dispersive X-Ray Spectroscopy/SEM-EDX (Hitachi High-Tech TM3000; Hitachi High-Technologies Corporation, Tokyo, Japan). Magnetic properties were observed by Electron Spin Resonance (Leybold Heraeus; Leybold, Huerth, Germany) at room temperature with electric current 0.3 A, coil radius and frequency were 6.8 cm and 33.7-76.5 MHz, respectively. Magnetic properties were proved by calculating the g-Landé factor of each sample nanoparticles. The lower g-Landé factor indicates higher magnetism of the nanoparticles. Further analysis by calculating the surface per volume ratio (\(K_s/K_v\)) was required to confirm magnetic properties data. \(K_s\) value represented the surface area, and \(K_v\) value represented of the formed magnetic nanoparticles. Higher \(K_s/K_v\) ratio represents a higher surface area of the formed magnetic nanoparticles, which reflects higher magnetic susceptibility. The \(K_s/K_v\) ratio value could be used to clarify the spin configuration of nanoparticles that affects their magnetism.\(^{16}\)

RESULTS AND DISCUSSION

Chitosan-Coated Fe$_3$O$_4$ Nanoparticles Synthesis

The magnetic of iron oxide coated by chitosan (chitosan-Fe$_3$O$_4$) nanoparticle were prepared using ex-situ co-precipitation method. The mechanism of ex-situ process to form chitosan-Fe$_3$O$_4$ nanoparticle consist of two-step reaction, which includes the formation of Fe$_3$O$_4$ nanoparticles prior to the formation of the final
product. All synthesis steps of chitosan-Fe$_3$O$_4$ nanoparticles by ex-situ co-precipitation method were illustrated in Fig.-2.

For the first step, alkaline condition was necessary for Fe$_3$O$_4$ nanoparticles formation. In this work, ammonia solution (3 mol L$^{-1}$) was used as an alkaline solution. It was purposed to control final pH of the solution and conducting a higher abundance of formed Fe$_3$O$_4$. Utilizing NaOH as a base solution might cause rapidly increasing the solution pH above 8 and difficult to control final pH of the solution, and potentially conducted formation of low magnetism compounds ($\alpha$-NaFeO$_2$). On the other hand, pH of the solution can increase gradually by using ammonia solution. This result was in agreement with the previous research by Yuanbi, et.al. In terms of magnetic nanoparticles preparation, the pH value of the iron solution was adjusted at 10 by adding alkaline solution. The formation step of Fe$_3$O$_4$ was initialized by the precipitation of Fe$^{3+}$ ion at lower pH level (pH 3-4) and followed by the precipitation of Fe$^{2+}$ ion at the higher pH level (pH 8-9). The Fe$^{3+}$ ions begin to co-precipitate with the Fe$^{5+}$ ion at pH 8.5, and the process of co-precipitation optimally occurred at pH > 9.2.

For the second step reaction, chitosan coated Fe$_3$O$_4$ were occurred based on an electrostatic interaction between the positively charged of chitosan with negatively charged around the surfaces of Fe$_3$O$_4$. Afterward, the ionic crosslinkers of TPP and sulfate were added to form chitosan networks which serve as a Fe$_3$O$_4$ coating to provide better biocompatibility and biodegradability of the nanoparticles. In addition, the combination of those crosslinkers contribute to establishing spherical morphology of chitosan-Fe$_3$O$_4$ nanoparticles. Interactions between the chitosan and ionic crosslinkers occurred through electrostatic interactions, which involve protonated of the amino group of chitosan and the negatively charged anionic group of TPP and sulfate. Nanoparticles were then dried and characterized to investigate the physical characteristics and magnetic properties of each material with various composition ratio and crosslinking time.

**XRD Pattern and Crystallite Size of Nanoparticles**

XRD patterns of a bare Fe$_3$O$_4$ and chitosan-Fe$_3$O$_4$ with different composition ratio and crosslinking time were depicted in Fig.-3. According to Inorganic Crystal Structure Database (ICSD) of Fe$_3$O$_4$ (ICSD #26410), six broad characteristic peaks of Fe$_3$O$_4$ with inverse spinel structure were attained. The peaks were observed around 2$\theta$ of 30°, 35°, 43°, 53°, 57°, and 62° that corresponded to Miller indices of (220),
(311), (400), (422), (511), and (440), respectively. Those broadening peaks indicate an existence of small size of nanoparticles. The ratio of chitosan:Fe\textsubscript{3}O\textsubscript{4}, and TPP:sulfate at various composition, resulted in similar diffractogram patterns with bare Fe\textsubscript{3}O\textsubscript{4} (Fig. 3a and 3b). However, with the increasing crosslinking time, over 1 hour crosslinking time (Fig.-3c), an additional peak was observed at 2\(\theta\) around 21.56\(^{\circ}\) which indicates chitosan peak with an orthorhombic structure (\(P 2_1 2_1 2_1\)) as also reported by Gong, et.al.,\textsuperscript{22} and Kawahara, et.al.\textsuperscript{23} It could be concluded that longer crosslinking time can cause chitosan to combine with other chitosan and built thicker chitosan matrix with larger hollow templates for Fe\textsubscript{3}O\textsubscript{4}. According to the ICSD data, it was found that Miller indices and 2\(\theta\) position of Fe\textsubscript{3}O\textsubscript{4} (ICSD #26410) and \(\gamma\)-Fe\textsubscript{2}O\textsubscript{3} (ICSD #172905) were almost identical as shown in Fig.-3.

Crystallite size of bare Fe\textsubscript{3}O\textsubscript{4} and chitosan-Fe\textsubscript{3}O\textsubscript{4} nanoparticles prepared at various parameters were listed in Table 1. Crystallite size decreased along with the increasing ratio of chitosan:Fe\textsubscript{3}O\textsubscript{4} as a result of Fe\textsubscript{3}O\textsubscript{4} distribution in chitosan solution. Hence, Fe\textsubscript{3}O\textsubscript{4} clusters are separated to form smaller size Fe\textsubscript{3}O\textsubscript{4}. The crystallite of Fe\textsubscript{3}O\textsubscript{4} nanoparticles can interact with chitosan to produce chitosan-coated Fe\textsubscript{3}O\textsubscript{4} nanoparticles.

![Fig.-3](image-url)

**Fig.-3:** X-ray diffraction patterns of both bare Fe\textsubscript{3}O\textsubscript{4} and chitosan-Fe\textsubscript{3}O\textsubscript{4} nanoparticles with different (a) chitosan:Fe\textsubscript{3}O\textsubscript{4} ratio, (b) TPP:sulfate ratio and (c)crosslinking time

| Parameter | %Fe\textsubscript{3}O\textsubscript{4} | %Fe\textsubscript{2}O\textsubscript{3} | Particle Size\(^a\)(nm) | \(L^b\)(nm) | \(K_s\) (nm\(^2\)) | \(K_v\) (nm\(^3\)) | \(K_s/K_v\) |
|-----------|----------|----------|----------------|--------|-------------|-------------|-------------|
| Bare Fe\textsubscript{3}O\textsubscript{4} | 87.98 | 12.02 | 72.3-162 | 11.90 | 444.655 | 881.899 | 0.504 |
| Ratio of Chitosan:Fe\textsubscript{3}O\textsubscript{4} | \| | \| | \| | \| | \| | \|
| 1:1 | 93.29 | 6.71 | 253-482 | 6.20 | 120.702 | 124.725 | 0.967 |
| 2:1 | 92.73 | 7.27 | 456-793 | 4.27 | 57.251 | 40.744 | 1.405 |
| 3:1 | 89.35 | 10.65 | 583-882 | 3.78 | 44.865 | 28.265 | 1.587 |

Table-1: Determination of particle size, crystallite size, and \(K_s/K_v\) value of coated-Fe\textsubscript{3}O\textsubscript{4} nanoparticles
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| Ratio of TPP:Sulfate | 1:1  | 3:1  | 5:1  |
|---------------------|------|------|------|
|                     | 89.88| 93.29| 96.30|
| Cross-linking Time (h)| 10.12| 6.71 | 3.70 |
|                     | 368-593| 253-482| 202-273|
|                     | 7.79 | 6.20 | 5.34 |
|                     | 190.548| 120.702| 89.538|
|                     | 247.395| 124.725| 79.689|
|                     | 0.770 | 0.967| 1.123|

Cross-linking Time (h)
| 1 | 93.29 | 6.71 | 253-482 | 6.20 | 120.702 | 124.725 | 0.967 |
| 2 | 94.52 | 5.48 | 283-320 | 8.75 | 240.406 | 350.592 | 0.685 |
| 3 | 97.12 | 2.88 | 142-226 | 9.44 | 279-816 | 440.245 | 0.635 |

*Measured by SEM instrument; Unstrained crystallite size using modified Scherrer Equation (1)*

Furthermore, crystallite size decreased with the increasing ratio of crosslinkers of TPP:sulfate. Tripolyphosphate is a crosslinker that can interact with chitosan by inter- and intra-molecular interactions, providing smaller crystallite size and spherical morphology of materials. The higher TPP:sulfate ratio, the smaller crystallite size chitosan-Fe₃O₄ nanoparticles produced. However, large crystallite size was obtained with a longer crosslinking time.

Crystallite size distributions were presented in Fig.-4. From three different composition ratio of chitosan:Fe₃O₄ as displayed in Fig. 4a, the most uniform crystallite size were obtained in the smallest ratio of chitosan:Fe₃O₄. Higher crystallite size homogeneity was attained at a lower chitosan composition.

![Crystallite size distribution](image)

**Fig.-4:** Crystallite size distribution of chitosan-Fe₃O₄ nanoparticles in different (a) chitosan: Fe₃O₄ ratio, (b) TPP:sulfate ratio and (c) crosslinking time

On the contrary, in agreement with Fig.-4b, the higher ratio of TPP and sulfate will lead to higher uniformity of the crystallite size. In this case, the crystallite size distribution was significantly affected by rearrangement of the chitosan template which was influenced by the amount of chitosan and crosslinkers. A large amount of chitosan will decrease the homogeneity of crystallite size distribution. Meanwhile, numerous amounts of crosslinkers will contribute to the increasing crystallite size distribution homogeneity, because chitosan template shrunk significantly and produced chitosan-Fe₃O₄ nanoparticles.
with more uniform crystallite size. On the other hand, Fig. -4c demonstrated the effect of crosslinking time to the crystallite size distribution. The longer crosslinking time, the bigger crystallite size with higher homogeneity obtained. According to the result above, the longer crosslinking time will lead to a better distribution of crosslinking agent and provide more uniform distribution of the crystallite size.

**Particle size, morphology, and magnetic properties of nanoparticles**

Surface morphology of bare Fe$_3$O$_4$ and chitosan-Fe$_3$O$_4$ nanoparticles in different crosslinkers ratio were observed by SEM and presented in Fig.-5.

![SEM images](image)

As shown Fig.-5, particles joint to form clusters that could be occurred during purification of Fe$_3$O$_4$ prior to chitosan coating. The coating process encouraged the spherical formation of chitosan-Fe$_3$O$_4$, as can be seen in Fig.-5. Crosslinker plays a significant role to establish better spherical morphology of nanoparticles. The particle size of both bare Fe$_3$O$_4$ and chitosan-Fe$_3$O$_4$ nanoparticles were summarized in Table-1.

Elemental analysis of bare Fe$_3$O$_4$ nanoparticles by EDX shows that bare Fe$_3$O$_4$ nanoparticles sample only has two main components, Fe and O. The percentages (w/w) of those components were 80.9% and 19.1% for Fe and O, respectively. The presence of chitosan and crosslinkers (TPP and sulfate) in the chitosan-coated Fe$_3$O$_4$ nanoparticles could be identified by the existence of C (16.5 – 20.3%), N (6.5 – 7.8%), P (1.4 – 1.7%), and S (0.011%).

Three peaks of EDX spectra for bare Fe$_3$O$_4$ nanoparticles existed at around binding energy of Fe (0.8, 6.3, and 6.8 KeV). However, to consider iron oxide phase that contained in various samples, it was necessary to further analysis using XRD pattern of the samples with MAUD software. As the results of iron oxide phase analysis, chitosan-Fe$_3$O$_4$ nanoparticles contained not only the Fe$_3$O$_4$ phase but also $\gamma$-Fe$_2$O$_3$. A crystal of Fe$_3$O$_4$ containing the mixtures of Fe$^{3+}$ and Fe$^{2+}$ atoms in octahedral sites with the same ratio, besides the Fe$^{3+}$ atoms in tetrahedral sites. This crystal form inverse spinel structure with $Fd\bar{3}m$ space group. In contrast, the crystal of $\gamma$-Fe$_2$O$_3$ was formed as an oxidation product of Fe$_3$O$_4$. The Fe$^{2+}$ atom in
octahedral sites was converted to Fe$^{3+}$ sites. As the result, iron vacancies appear on this site and the structure converts as defect spinel structure.

The presence of chitosan in small concentration will prevent oxidation of Fe$_3$O$_4$ into $\gamma$-Fe$_2$O$_3$. However, the excess chitosan might be able to reduce Fe$_3$O$_4$ content as well as reduce magnetism. The thicker chitosan matrix caused the weaker magnetism because this matrix would impede interaction between magnetic nanoparticles with external magnetic field. Therefore, the higher external magnetic field would be required to control the chitosan-Fe$_3$O$_4$ nanoparticles.

There was a correlation between crystallite size, $g$-Landé factor, and Fe$_3$O$_4$ content for nanoparticles samples with different composition ratio and crosslinking time as depicted in Fig.-6. The presence of chitosan conducted better formation of nanoparticles and higher abundance of Fe$_3$O$_4$ than bare Fe$_3$O$_4$, because chitosan prevented oxidation process of Fe$_3$O$_4$ into $\gamma$-Fe$_2$O$_3$. The presence of chitosan also induced the crystallite size of Fe$_3$O$_4$ significantly to become smaller. So that, it can be classified as high field resonance material category (Fig.-6a and Fig.-7). However, Fe$_3$O$_4$ magnetism would be reduced slightly while chitosan added in small concentration (for chitosan:Fe$_3$O$_4$ from 1:1 to 2:1); and would be reduced significantly and classified as low magnetic resonance materials while higher concentration added (for chitosan:Fe$_3$O$_4$ from 2:1 to 3:1). This phenomenon explained that thicker chitosan layer would obstruct magnetic interaction between external magnetic field and Fe$_3$O$_4$, even though there were high Fe$_3$O$_4$ content, small crystallite size, and high magnetic susceptibility of Fe$_3$O$_4$.

![Fig.-6: Correlation curve between $g$-Landé factor toward crystallite size and percentage of Fe$_3$O$_4$ in chitosan-Fe$_3$O$_4$ samples with different (a) chitosan:Fe$_3$O$_4$ ratio, (b) TPP:sulfate ratio and (c) crosslinking time](image)

Longer crosslinking time exhibited inverse results than different composition ratio, which were increasing trends of a $g$-Landé factor, Fe$_3$O$_4$ content, and crystallite size (Fig.-6c and Fig.-7). These trends gave further information that longer crosslinking time conducted chitosan re-arrangement to form thicker chitosan matrix and larger hollow templates of Fe$_3$O$_4$ as occurred in samples with the higher chitosan concentration. Formed thicker chitosan matrix through longer crosslinking time exhibited lower magnetism because the formed matrix obstructed interaction between the formed Fe$_3$O$_4$ with external magnetic field even though had higher Fe$_3$O$_4$ content. This reason was supported by larger crystallite size of Fe$_3$O$_4$ which reflected lower magnetic susceptibility. Therefore, the high external magnetic field would be required to accompany the formed Fe$_3$O$_4$ as a drug-delivery system. From these results, we assumed that the influence of each parameter towards crystallite size, magnetic phase abundance, layer thickness, and magnetism of chitosan-Fe$_3$O$_4$ nanoparticles has been summarized in Fig.-7. This schematic summary illustration was made to simplify understanding of the phenomenon that attained from the data of crystallite size, magnetic phase abundance, and magnetic properties of nanoparticles.
Smaller crystallite size indicates the higher surface per volume ratio ($K_s/K_v$), as given in Table-1. In this case, the higher $K_s/K_v$ value exhibited the higher magnetism because of de-orientation of electron spin direction. Even though the crystallites have higher $K_s/K_v$ which provided higher magnetism, the higher magnetic competition of each crystallite also occurred because of higher distribution of defragmented crystallites.\textsuperscript{24} The effect of various parameters towards de-orientation of electron spin direction, magnetism, magnetic competition, and g-Landé factor could be illustrated in Fig.-8. The increasing of chitosan composition conducted smaller crystallite size which indicated higher magnetic susceptibility, magnetic competition, and spin de-orientation. The presence of thin chitosan layer prevented oxidation of $\text{Fe}_3\text{O}_4$ and increasing their magnetic susceptibility, so their magnetism became stronger (g-Landé factor of 1.785). However, as explained by previous discussion (Fig.-6a and Fig.-7), thicker chitosan layer would greatly reduce their magnetic interaction with external magnetic field, so their magnetic susceptibility became ineffective. These arguments were supported by their weak magnetism (g-Landé factor $\geq$ 2.000), and higher magnetic moment competition with other coated-$\text{Fe}_3\text{O}_4$ crystallites (as illustrated in Fig.-8).

Fig.-7: The influence of each various parameter towards structural and magnetic properties, and its formation mechanism of synthesized chitosan-$\text{Fe}_3\text{O}_4$ nanoparticles.

The similar phenomenon was also occurred by tuning crosslinking time. The smaller crystallite size was obtained with short duration of crosslinking time, that represented higher magnetic susceptibility and it was also supported by their stronger magnetism (g-Landé factor of 1.785). Longer crosslinking time induced crystallite reconstruction which conducted larger crystallite size formation of $\text{Fe}_3\text{O}_4$ and chitosan layer. This phenomenon gave larger $\text{Fe}_3\text{O}_4$ crystallite, which provided less magnetic moment competition and spin de-orientation. Despite that, their magnetic properties could not be greater than nanoparticle
produced by the short duration of crosslinking time, because the reconstruction of chitosan layer (to form a thicker layer) would also obstruct magnetic interaction with the external magnetic field. Hence, the nanoparticles became less responsive to the external magnetic field. Another phenomenon occurred for samples with a different composition ratio of crosslinkers. The increase of TPP:sulfate ratio produced smaller crystallite size and higher Fe₃O₄ content, however, they have lower magnetism. In this case, trends of magnetism did not follow the increasing trends of TPP:sulfate ratio. It was theoretically hard to explain the phenomenon, so further investigation might be required to explain this observable fact.

CONCLUSION
Magnetic nanoparticles of Fe₃O₄ coated by chitosan as a candidate of drug delivery agent were successfully obtained by ex-situ co-precipitation method. Surface modification by chitosan (in small concentration) was employed to prevent oxidation of Fe₃O₄ into γ-Fe₂O₃. The combination of tripolyphosphate and sulfate as crosslinking agents were also required to produce the smaller size of chitosan-Fe₃O₄ particles with spherical morphology. Physical characteristics and magnetic properties of chitosan-Fe₃O₄ nanoparticles were observed in detail. Crystallite size of chitosan-Fe₃O₄ nanoparticles was evaluated which was less than 10 nm. According to XRD, SEM, and ESR data, there was a correlation between Fe₃O₄ content, crystallite size, and g-Landé factor. For nanoparticles samples with different
chitosan content and different crosslinking time, smaller crystallite size will lead to the decreasing Fe₃O₄ content and result in low magnetism. But another phenomenon occurred in samples with different crosslinkers ratio and further observation would be required to explain in more detail.

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