UNIVERSITI PUTRA MALAYSIA

BIOGENIC AND CHARACTERIZATION OF IRON OXIDE NANOPARTICLES (IONPs)

AKMAL ARIF BIN NASRULDIN

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BY

AKMAL ARIF BIN NASRULDIN

198134

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DEDICATION

This thesis is dedicated to:

My supervisor, Assoc. Prof. Dr. Che Azurahanim Che Abdullah

My beloved parents, Nasruldin Bin Othman and Norzaila Binti Abdul Latiff

My project coordinator, Emmelie Laura

My lecturers,

My siblings,

My friends,

and others who help me through the completion of this thesis.

Thank you for the help, support, love, and encouragement.

ABSTRACT

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Supervisor: Assoc. Prof. Dr. Che Azurahnim Che Abdullah

Faculty: Faculty of Science, Universiti Putra Malaysia

Green nanoparticle synthesis is a new method for making nanoparticles from biological materials. It is getting more popular because it is low-cost, environmentally friendly, and can be can be produced on a large scale. This research project reports a green synthesis approach for the preparation of iron nanoparticles (IONPs) using roselle flower extract (*Hibiscus sabdariffa*). The synthesis of IONPs was carried out by mixing an aqueous solution of the roselle flower extract (*Hibiscus sabdariffa*) and iron oxide (IONPs) solution. The factors affecting synthesis were identified and optimised using Taguchi. The synthesised IONPs are characterised by various techniques, including UV-vis spectrophotometer (UV-VIS) and Fourier transform infrared spectroscopy (FTIR). The optimal conditions for IONPs synthesis were determined according to Taguchi experimental procedures as the temperature equal to 70^{o}C, 80^{o}C and 90^{o}C time equal to 1 hours, and a ratio of Ammonia: plant extract in solution of 1:1, 1:3 and 1:9. The absorption maxima of the synthesised IONPs showed a characteristic of iron

surface plasmon resonance (SPR) peak at 260 nm by UV-Vis analysis. Finally, FTIR analysis has provided information on the presence of numerous bioactive compounds that are responsible for the stabilization and capping of the IONPs.

ABSTRAK

BIOGENIK DAN CIRI-CIRI NANOPARTIKEL BESI OKSIDA (IONPs)

OLEH

AKMAL ARIF BIN NASRULDIN

198134

FEBRUARI 2022

Penyelia: Prof. Madya Dr. Che Azurahanim Che Abdullah

Fakulti: Fakulti Sains, Universiti Putra Malaysia

Sintesis nanozarah hijau ialah kaedah baharu untuk membuat zarah nano daripada bahan biologi. Ia semakin popular kerana kos rendah, mesra alam, dan boleh dihasilkan secara besar-besaran. Projek penyelidikan ini melaporkan pendekatan sintesis hijau untuk penyediaan nanozarah besi (IONPs) menggunakan ekstrak bunga rosella (*Hibiscus sabdariffa*). Sintesis IONPs dijalankan dengan mencampurkan larutan akueus ekstrak bunga rosella (*Hibiscus sabdariffa*) dan larutan oksida besi (IONPs). Faktor yang mempengaruhi sintesis telah dikenalpasti dan dioptimumkan menggunakan Taguchi. IONP yang disintesis dicirikan oleh pelbagai teknik, termasuk Spektroskopi Ultraungu-Kelihatan (UV-VIS) dan Spektroskopi Transformasi Fourier Inframerah (FTIR). Keadaan optimum untuk sintesis IONPs ditentukan mengikut prosedur eksperimen Taguchi kerana suhu bersamaan dengan masa 70^{o}C, 80^{o}C dan 90^{o}C bersamaan dengan 1 jam, dan nisbah Ammonia: ekstrak tumbuhan dalam larutan 1:1, 1:3 dan 1:9. Maksimum penyerapan IONPs yang disintesis menunjukkan ciri puncak

resonans plasmon permukaan besi (SPR) pada 260 nm oleh analisis UV-Vis. Akhir sekali, analisis FTIR telah memberikan maklumat tentang kehadiran banyak sebatian bioaktif yang bertanggungjawab untuk penstabilan IONPs.

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In the name of Allah. the most Beneficent, the most Merciful. Praise is to Allah who gave me the strength. the power, the motivation, help and patience to complete this project and study after so many hurdles and obstacles; May blessing and peace be upon our prophet Muhammad (S.A.W).

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I love you guys.

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LIST OF ABBREVIATIONS

MNPs	Metal Nanoparticles
IONPs	Iron Oxide Nanoparticles
NaOH	Natrium Hydroxide
SPR	Surface Plasmon Resonance
UV-Vis	Ultraviolet-Visible Spectroscopy
FTIR	Fourier Transform Infrared Spectroscopy
$FeCL_2.4H_2O$	Iron (II) Chloride Tetrahydrate
$FeCL_3.6H_2O$	Iron (III) Chloride Hexahydrate
NH_4OH	Ammonium hydroxide
HCL	Hydrochloric acid
DI	Distilled
λ	Wavelength
XRD	X-Ray Diffraction
TEM	Transmission Electron Microscopy
Fe^{3+}	Iron ion
Fe^{0}	Iron atom
VSM	Vibrating Sample Magnetometer
FESEM	Field Emission Scanning Electron Microscope

CHAPTER 1

INTRODUCTION

1.1 Nanotechnology

Nanotechnology is one of the most study fields in science such as material science, as well as one of the disciplines of science and engineering. It is a capacity to develop new structures at the atomic level size, which range from 1 to 100 nm . (Silvestre et al., 2016). Fullerenes, metal, ceramic, and polymeric nanoparticles are among the many types of nanoparticles.

Iron oxide nanoparticles (IONPs) are nanoparticles that used in medical applications. IONPs are easy to prepare, solubility, and low toxicity, and the ability to regulate the growth of nanoparticles. (Clarke et al. 2017). It is a superparamagnetic nanoparticle with unusual characteristics. Superparamagnetic is a kind of magnetism seen in ferromagnetic and ferrimagnetic nanoparticles. (Weinstein et al., 2009). Superparamagnetic is important in biomedical applications since the particles are well dispersed in a liquid, such as water. Also, for organic or inorganic material to produce composites. Superparamagnetic are better than that ordinary paramagnetic of materials due to their stronger properties. Also, it may approach the saturation point of ferromagnetic iron oxide. This feature allows these molecules to be tracked in a magnetic field without affecting the colloidal solution's stability. Furthermore, the Neel relaxation process may be used to measure the heat of the particles by supplying a changing magnetic field. (Neuberger et al., 2005).

IONPs can be synthesis using various method. Firstly, physical method such as laser ablation, electron beam lithography, laser ablation, powder ball milling and aerosols. Secondly, chemical method like co-precipitation, hydrothermal, microemulsion, electrochemical deposition, sonochemical and thermal decomposition. Also biological method that using fungi, bacteria, algae and plant (Abbas & Krishnan, 2020).

IONPs can be used in wide range biomedical application such as hyperthermia. Hyperthermia is the use of an oscillating magnetic field to raise the temperature of tumour cells. It's one of the most potential non-invasive cancer treatments since it can kill cancer cells without harming healthy cells. This is because tumours are more sensitive than normal cells to temperature elevation. The intracellular injection of high temperatures (often between 41–46 ^{o}C) can cause breakdown mechanisms. Next, IONPs can be used in drug targeting delivery. Medication targeting has developed as a viable method of drug delivery in the contemporary era. It is one of the techniques that uses IONPs to transport particles to the correct target area. (Kalubowilage et al., 2019).

Table 1.1 Method to synthesis IONPs

Method to synthesis IONPs	**Method**
Physical Method	Laser ablation, gas phase deposition, electron beam lithography, laser ablation, powder ball milling and aerosols
Chemical Method	Co-precipitation, hydrothermal, microemulsion, electrochemical deposition, sonochemical and thermal decomposition
Biological Method	Fungi, bacteria, algae and plant

1.2 Problem statement

The conventional method to synthesis IONPs has many drawbacks. Some methods have poor dispersion and uniformity of size and particle distribution. Example of these process are hydrothermal method, co-precipitation, and sol–gel processes. Chemical methods like these can produce toxic waste that are harmful to both humans and the environment. It is because chemical methods have harmful contents such as toxic metals, radiation and viruses.(Khalil et al., 2017).

This problem can be overcome by using the green synthesis method. A green method is environmentally friendly that uses plant extracts as reducing and stabilizing agent to reduce the size of nanoparticles and prevent aggregation (Ahmad et al. 2019). Environmentally friendly nanoparticle manufacturing processes provide a variety of benefits over chemical methods. It is because the green method does not need external conditions such as energy, high pressure and the use of hazardous chemicals which results in significant energy savings. Finally, the benefits of the green method are long-term since it is a good process that reduces the production of hazardous chemicals. (Herlekar et al. 2014).

A green IONPs have been successfully synthesised using aloe vera and its was discovered that it has the average size of around 50 nm with superparamagnetic it has potential to be used in Magnetic Resonance Imaging (MRI) applications (Rahmani et al., 2020). Similarly, another research has successfully synthesized IONPs using hibiscus rosa for fortifying wheat biscuits (Razack et al., 2020). Another study manages to use Mongostana fruit peel extract to fabricate IONPs and it has an average size of around 13 nm which make its superparamagnetic. It has the potential to be used in hyperthermia application (Webster & Kuča, 2021). This demonstrates that the green approach may be utilised in a range of medical and nutritional applications since it is less hazardous and does not damage the environment.

In this project we want to fabricate IONPs using green methods since they are low cost, eco-friendly and sustainable. The plant of interest in these studies is Roselle as shown in Figure 1.1. The roselle flower (Hibiscus sabdariffa L.) is a natural source of antioxidant-rich red–purple chemicals. Roselle is cultivated for it's own calyces, which are sold from Sudan, China, and Thailand, as well as Mexico. It is collected by nomadic goat-herding tribes in Sudan, but the product is often inferior owing to poor processing conditions. Nonetheless, the Sudan product is vibrantly red, very acid, and wildly popular in German, which imports the majority of the crop. Diarrhea, dysentery, hypertension, hypercholesterolemia, and urinary tract infections are all treated with roselle juice. Because of its high phenolic content (mostly anthocyanins), polysaccharides, and organic acids. Hence, this research plan to synthesized IONPs using roselle in an attempt to produce better IONPs. (Ochoa-Velasco & Ruiz-López, 2019). Hence, this research plan to synthesized IONPs using roselle in an attempt to produce better IONPs than its chemically produce counterpart.

Figure 1.1 Roselle flower (Source: https://www.rhymbahillstea.com/health-benefits-roselle).

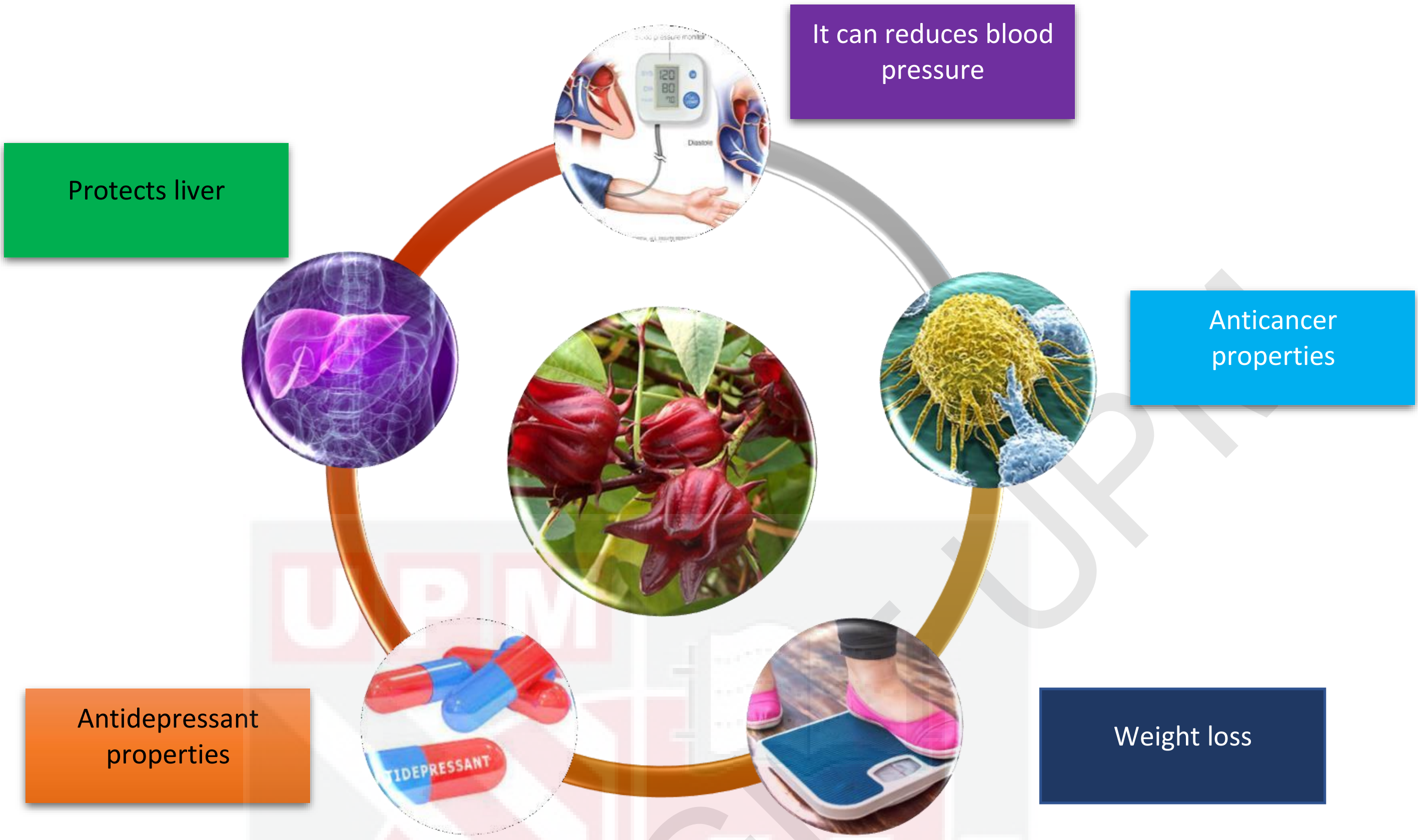


Figure 1.2: The benefits of Roselle Flower

1.3 Objectives

The main objectives of this project are:

1) To optimize synthesis parameter of Iron Oxide Nanoparticle (IONPs) by using Taguchi method.
2) To synthesis biogenic IONPs by using Roselle flower extract.
3) To characterize the chemical bonds and optical properties of IONPs by using FT-IR and UV-Vis.

CHAPTER 2

LITERATURE REVIEW

This chapter conduct a review of the literature pertinent to the current research. This chapter is divided into three parts. The first section is the general introduction of IONPs and their unique properties. The second part discusses the synthesis process of IONPs chemical method specifically co-precipitation and green method. The third portion review the benefits of utilising IONPs in biomedical applications, notably targeted drug administration, and is followed by an explanation of IONPs magnetic characteristics, particularly their superparamagnetic capabilities, in the fourth section. The fifth section discuss about the taguchi method.

2.1 General Introduction of Iron Oxide Nanoparticles and Their Unique Properties

IONP (Fe_3O_4) exhibits remarkable electrical and magnetic properties. This is because electron transfer occurs between Fe^{2+} and Fe^{3+} in the octahedral. IONP is widely used in various technical applications. Examples are catalysts, ferrofluids, energy storage, bioprocessing and data storage. IONPs are made in a variety of ways depending on their intended application. For example, demonstrates that while the magnetic cores of IONPs include metals such as cobalt (Co), nickel (Ni), and neodymium-iron-boron (NIB), they are prone to oxidation and therefore dangerous to the human body. (Hu et al., 2006)

Nanoscience is a rapidly growing area of study in contemporary science. One example of nanoscience is nanotechnology. Nanotechnology is currently used in pharmaceutical, medical, electronics, robotics, and tissue engineering. Nanoparticles have provided many advantages due to their unique size and physical properties. For example, an ability to connect to functional molecules on a selective basis.

There are many variables that influence the serious size of a single domain in terms of magnetic domain theory. For example, magnetic saturation values, crystal anisotropy, exchange forces, particle shape, and surface. When a ferromagnetic material is imposed on a magnetic field, the reaction is indicated by a hysteresis loop. It consists of two different components: remanence and coercivity. Remanence is the magnetism that is inside the ferromagnetic material after an electromagnetic field is attracted. Magnetic materials' remanence provides magnetic memory for magnetic storage devices. So, coercivity is proportional to the curve's thickness. When a particle size decreases, coercivity rises to the largest value before falling to zero.

The coercivity of a superparamagnetic particle is zero when its size is lower below the critical level. Additionally, thermal factors contribute to the formation of superparamagnetism. Thermal fluctuations in superparamagnetic particles are strong enough to demagnetize a saturation spontaneously. Thus, these particles exhibit no coercivity or hysteresis as demonstrated by well as (Willard et al., 2004).

When an external magnetic field is applied, the nanoparticle gets magnetised. But, when the external magnetic field is removed, the nanoparticle becomes demagnetized. As a result, the active behaviour of the particle is prevented. When IONPs are introduced into a living system, they become magnetised only under the influence of certain magnetic fields, giving them the particular properties needed for biological applications.

2.2 The physical and magnetic properties of IONPs

The properties of IONPs can be separated into two parts such physical properties and its magnetic properties.

2.2.1 Physical properties of IONPs

IONPs have a small size ranging from 1-100 nm. The work done by Razack et al., (2020), managed to obtain the average size of 6 nm with spinel shape and ferromagnetic properties and from Gahrouei et al., (2020) obtain the average size of around 10-40 nm and the shape of IONPs is spherical shape with antiferromagnetic properties. Another research from Puscasu et al., (2016) find that IONPs have the size of around 15nm with the ferromagnetic properties and spherical shape by using so-gel method. Then Yadav et al., (2020) obtain that the size of around 39 nm Fe_3O_4 by using sonochemistry method with spherical shape and diamagnetic properties. Research done by Patsula et al., (2016) have successfully synthesize Fe_3O_4 with the average size of around 10-24 nm with spherical shapes and ferrimagnetic properties by using thermal decomposition method and the size of around 14 nm with spherical shapes and ferrimagnetic properties by using co-precipitation method. Besides that, from Yew et al., (2016) stated that the size of iron oxide has the size of around 15 nm with paramagnetic properties and spherical shapes by using green co-precipitation method.

Table 2.1 Comparison between Fe3O4

Average size (nm)	Shape	Properties	Method	Author
6	Spinel	Ferromagnetic	Co-precipitation	Razack et al., (2020),
10-40	Spherical	Antiferromagnetic	Co-precipitation	Gahrouei et al., (2020)
15	Spherical	Ferromagnetic	So-gel	Puscasu et al., (2016)
39	Spherical	Diamagnetic	Sonochemistry	Yadav et al., (2020)
10-24	Spherical	Ferrimagnetic	Thermal decomposition	Patsula et al., (2016)
14	Spherical	Ferrimagnetic	Co-precipitation	Patsula et al., (2016)
15	Spherical	Superparamagnetic	Green co-precipitation	Yew et al., (2016)

2.2.2 The magnetic properties of IONPs

Magnetic materials exhibit magnetism due to the mobility both atoms and electrons. There are two types of motion: orbital and spin. Magnetic moment has an impact on each of these motions. The orbital motion of an electron occurs when it rotates around the nucleus of an atom. While the moment of rotation occurs when an electron rotates around its axis. Then, the total of these two moments is referred to as the net magnetic moment. It demonstrated that an atom's completely occupied electron subshells have no impact on the atom's magnetic moment, since the atom has no net magnetic moment.

Magnetism is classified into two types: ferromagnetism and diamagnetism. Ferrimagnetism and anti-ferromagnetism are two different types of ferromagnetism. All materials have one of these forms of magnetism, which is categorized based on how they act to an external magnetic field

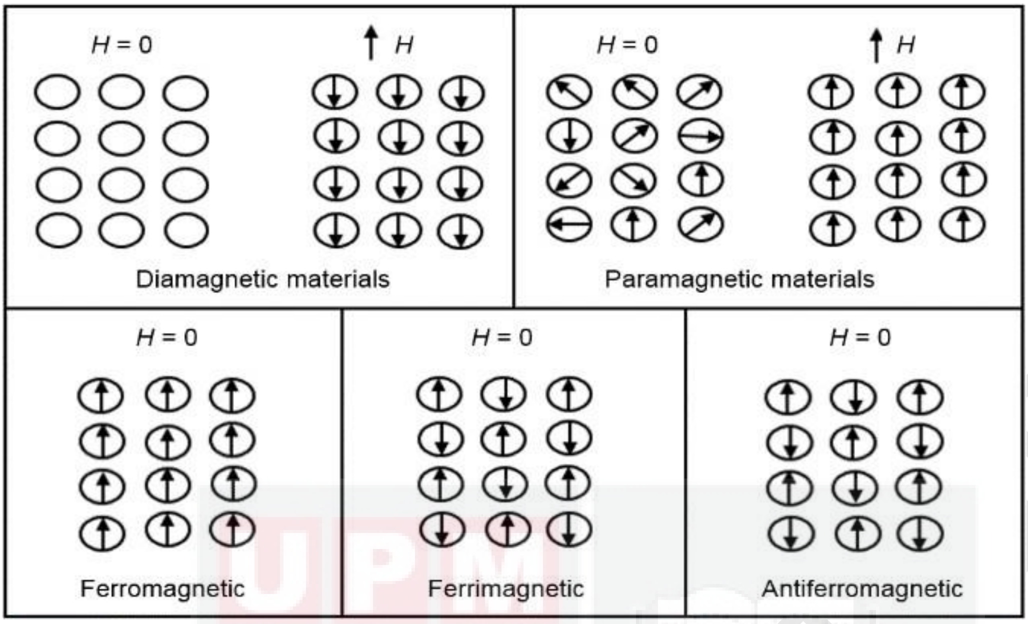


Figure 2.1 Magnetic dipoles arrangements for different types of materials in the presence or absence of an external magnetic field

Types of magnetism are described below:

Diamagnetism: The existence of a magnetic field causes the electron of diamagnetic materials to exist. As a result, it produces an antiparallel magnetic field to the outside magnetic field, as seen in Figure 2.2. Furthermore, they create modest induced dipoles in the presence of a magnetic field.

Paramagnetism: When a magnetic field is removed, the electron spin of these materials has no particular direction (Figure 2.2). As a result, the net magnetic moment in any direction is zero. When a magnetic field exists, however, the electron spins will align themselves with the magnetic field, as illustrated in Figure 2.2.

Ferromagnetism: It is magnetic by nature, even when there is no external magnetic field. The magnetic dipoles are positioned next to each other. Ferromagnetic materials have a weaker magnetic dipole when there are no external magnetic fields. In addition, the magnetic dipoles are antiparallel and have different magnitudes.

Epherre et al. (2011) showed that when there is no external magnetic field, antiferromagnetic materials exhibit antiparallel adjacent dipoles. Then, their magnetism cancels out Figure 2.3 shows the behavior of different types of magnetism, where H is the strength of magnetic field, M is the magnetization, Mr is the remanent magnetization (the residual magnetization at zero applied field strength), and Hc is the coercive field (the external field required to decrease the magnetization back to zero). In Figure 2.3, ferromagnetic materials have hysteresis loop with Hc and Mr. Furthermore, it has a higher magnetic saturation compared to paramagnetic materials.

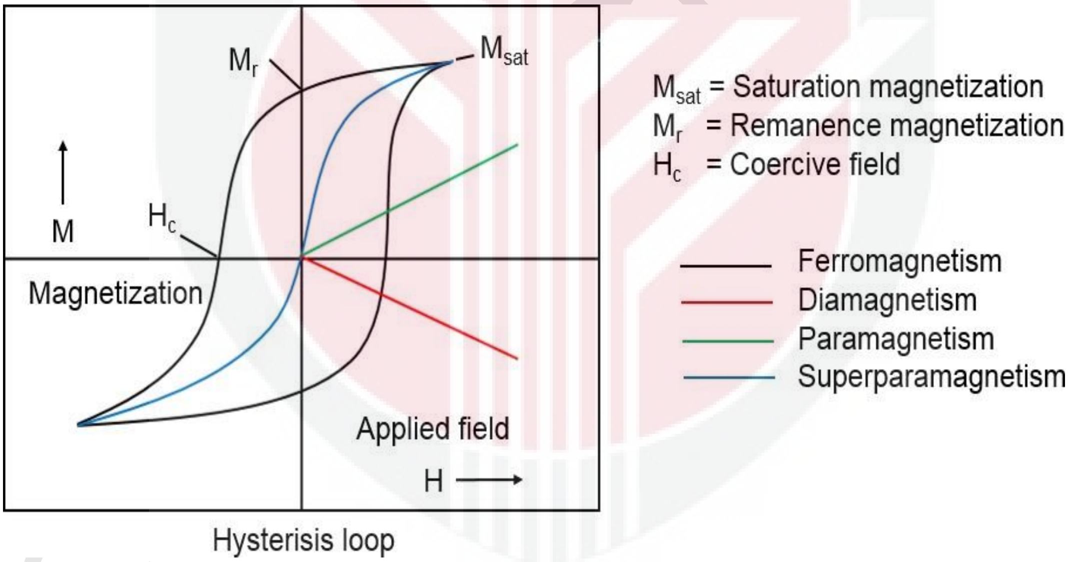
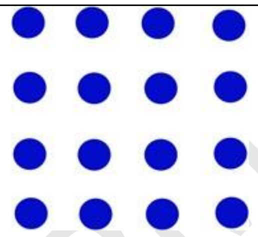
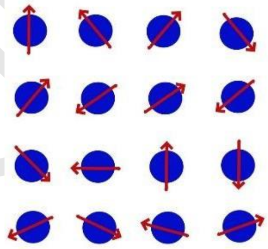
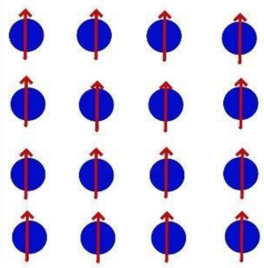
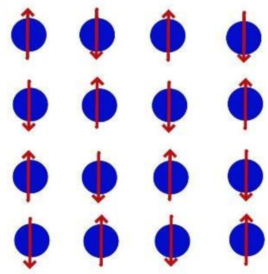
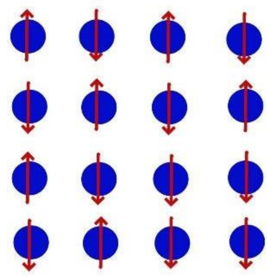


Figure 2.2 Magnetization curve for different types of magnetic behavior when magnetic field applied.

Table 2.2 Different between the type of magnetic properties

Type	**Example**		**Magnetic behaviour**
Diamagnetic	Inert gases; He, Ne, Ar, Kr, Xe, and Rn Diatomic molecule; H_2O, H_2, N_2	The magnetic moment of an atom is zero. The degree of susceptibility is low and negative. -10^{-6} to -10^{-5} is the range of possible values.	
Paramagnetic	Iron oxide beta phase; β-Fe_2O_3	Magnetic moments in atoms are arranged in a random pattern. Susceptibility is low and positive, ranging from $+10^{-5}$ to $+10^{-3}$.	
Ferromagnetic	Iron, nickel and cobalt.	Magnetic moments in atoms are oriented in parallel. Susceptibility is rather high (below T_C)	
Antiferromagnetic	Hematite; α-Fe_2O_3 Wüstite; FeO	Magnetic moments in atoms are antiparallel aligned. Susceptibility is low and positive, ranging from $+10^{-5}$ to $+10^{-3}$.	
Ferrimagnetic	Magnetite; Fe_3O_4 Maghemite; γ-Fe_2O_3	Magnetic moments aligned parallel and antiparallel in atoms are combined. Susceptibility is rather high (below T_C).	

2.2.3 Superparamagnetic

The magnetic behaviour changes when a multidomain becomes a single state domain when the particle size is lowered to a certain level. This is referred to as superparamagnetic behavior. Superparamagnetic materials are more helpful in the drug delivery sector because it can be magnetized and demagnetized quickly (Chomoucka et al., 2010). As a result, Arruebo et al. (2007) suggest that aggregation of the nanoparticles may be avoided.

Additionally, they lack hysteresis and coercivity, which are characteristics of paramagnetic materials (Chomoucka et al., 2010). But, for paramagnetic materials, this property is present only at a certain temperature. They, like ferromagnetic materials, have a high magnetic saturation value.

Moreover, superparamagnetic particles producing heat when exposed to an alternating current magnetic field. After that, the temperature in the surrounding region is increasing to 42-46 ^{o}C. In comparison to healthy cells, cancer cells are more susceptible to heat. As a result, this capacity is used in the medical sector to destroying cancer cells. When a cancer cell is exposed to a high temperature, it will be destroyed (Kumar et al., 2011). This is referred to as the hyperthermia technique.

2.3 Synthesis of IONPs

IONPs can be synthesized using chemical method specifically co-precipitation method and green method.

2.3.1 Chemical Method

The chemical method is one of the widely used synthesized methods of IONPs. One of the research done to synthesis IONPs are using the co-precipitation method. It is because, as detailed in many papers, co-precipitation is a simple technique for generating NPs such as Fe_3O_4. In the first step, 11.68 g $FeCl_3.6H_2O$ and 4.30 g $FeCl_2.4H_2O$ were dispersed in 200 ml DI water using an ultrasonic bath at 85 °C for 10 minutes under nitrogen gas. The pH was adjusted to 9 by continuously adding 1.0 M of freshly prepared NaOH solution. Then, the mixture was added 20 ml of a 30% ammonium solution to create the dark precipitate and the coloring was altered from orange into black. The particles of magnetite were washed twice and once using 0.01M hydrochloric acid (HCl). Magnetic NPs were heated at 80 °C for 4 h. The sample was collected and characterized using X-Ray Diffraction (XRD), Transmission Electron Microscopy (TEM), and Vibrating Sample Magnetometer (VSM). From the XRD results, the type of IONPs is Fe_3O_4 with an average size of around 22.3 nm. The potential application for the nanoparticles is drug delivery (Gahrouei et al., 2020).

Another study also synthesized IONPs by using co-precipitation. They manage to prepare Iron salt solutions which are $FeCl_3.6H_2O$: $FeCl_2.4H_2O$ with the molar ratios of 2:1 and 3:2. Each salt was combined with 25 ml distilled water in the necessary proportions to achieve molar ratios of 2:1 and 3:2 in a glass beaker. Then, the value of pH was adjusted to 11 by adding 0.5 M NaOH. The chloride solution was added to the ammonia solution until black precipitation was obtained. The temperature was stabilized at 70 ^{o}C. After that, the chloride solution was added to the ammonia solution until a black precipitate was produced by stirring for 30 minutes. Then, to remove the residual chlorides, the precipitate was repeatedly rinsed with distilled water. At room temperature, the IONPs were dried by air. The IONPs obtained are Fe_3O_4 with an average size of around 8 nm and 12 nm. The potential application for this IONPs is hyperthermia (Macías-martínez et al., 2016).

A separate study using the co-precipitation method also had successfully obtained Fe_3O_4 nanoparticles. About 10.14 g of $FeCl_3.6H_2O$ and 7.45 g of $FeCl_2.4H_2O$ were dissolved in water (25 ml). Then, ammonium (25%) was added into the salt solution under stir conditions at 700 rpm for 2 minutes. The value of pH was adjusted to 11 by adding 0.5 M NaOH. After that, 15 mL of the mixture was put in a Teflon-lined stainless autoclave, which was then heated in an oven for 12 h at 60 °C. The autoclave was cooled down to room temperature, and the precipitates were rinsed with tap water and separated using a magnet. After drying the final products were characterized. The nanoparticles have an average size of around 9 nm. It can be used in hyperthermia, MRI, magnetic cell separation, and drug delivery. (Jesus et al., 2020) . Table 2.1 showed the summary of particle size for IONPs and their potential application.

Table 2.3 The chemically synthesis IONPs particle size and its potential application

Particle size (nm)	pH	Applications	References
22.30	9	Drug delivery	(Gahrouei et al., 2020).
8-12.00	11	Hyperthermia	(Macías-martínez et al., 2016).
9.00	10	Hyperthermia, MRI, magnetic cell separation and drug delivery	(Jesus et al., 2020)

2.3.2 Green Method

Green synthesis, which uses plant extracts to synthesise IONPs, has been increasingly popular. Hibiscus is one of the plants used to synthesized IONPs. About 4.34g of $FeCl_3.6H_2O$ and 2.78g of $FeCl_2.4H_2O$ solution at a ratio of 2:1 was mixed in 160ml distilled water. The pH was adjusted to 12 by continuously adding 0.5 M of freshly prepared NaOH solution. Then, plant extract was added in the solution. The solution was continuously stirred for 1 h at 200 rpm at room temperature. After that, the solution is centrifuge at 7000 rpm for 15 minutes as many as 3 times to remove contaminants. The pellet was baked in a hot air oven at 40 °C overnight to remove moisture and undesirable particles. The type of IONPs that this project got is Fe_3O_4 with the average size of around 6.19 nm. The potential application for this IONPs are for fortifying wheat biscuits (Razack et al., 2020).

Another project uses aloe vera leaf extract to make IONPs. The leaf extract was cleaned before the extraction process. 3.0 g of the white part of the aloe vera leaf was immersed in 120 ml of distilled water for 6 h while boiling and stirring the liquid constantly. The extracted material was filtered and kept at 4 °C in a refrigerator. The $FeCl_3.6H_2O$ (9.36 g) and $FeCl_2.4H_2O$ (4.96 g) were also mixed in a 2:1 ratio solution. After that, a 150 ml of DI Water was mixed in a continuous stirred at a speed of 300rpm. The pH was adjusted to 11 by continuously adding 0.5 M of freshly prepared NaOH solution. The solution's colour darkens indicative of Fe_3O_4 formation. After stirring for 1 h the precipitate was centrifuged three times, and dried. The IONPs were cleaned and dried at a vacuum oven temperature of 70 °C for 24 h. The average size of the IONPs is around 30 nm and the potential application are therapeutic applications. (Rahmani et al., 2020).

Tangerine peel extract can also be used to synthesis IONPs. The plant was washed to remove surface contaminants and dried at 27 ^{o}C. The dried peelings were milled into powder. 50 g of granules were heated for 15 minutes at 80 °C in 500 ml distilled water. The plant extract was then allowed to cool to room temperature before being filtered using 0.45 filter paper. The plant extract was stored in refrigerator until further uses. The extract (500 ml) was mixed with 5.35 g of $FeCl_3.6H_2O$ and 8.10 g of $FeCl_2.4H_2O$ at different concentration of extract peel to produce 0.1 M solutions. The extracts peel act as surfactants and stabilizers. Then, stir the solution for 60 ^{o}C at 2 h. Finally, NH_4OH (25%) solution was added to this solution for at least 20 minutes, until the final pH was more than 9. A magnetic piece was used to capture the brown precipitate, which was then washed three times with distilled water. The treatment precipitant was dried in the laboratory for 1 week at room temperature. The IONPs have average size of around 50 nm and its had the potential to be used as water filter to remove cadmium ions in the water. (Ehrampoush et al., 2015).

Table 2.4 The green synthesis of IONPs particle size and potential application

Plants	Parts	pH	Particle size (nm)	Applications	References
Hibiscus Rosa Sinesis	Flower	12	6.19	Fortifying wheat biscuits	(Razack et al., 2020)
Aloe Vera Leaves	Leaf	11	14.00	Magnetic Resonance Imaging (MRI) and Hyperthermia	(Bibi et al., 2019)
Tangerine Peel	Fruit	9	50.00	Remove Cadmium ions from contaminated solution	(Ehrampoush et al., 2015)

2.4 Biomedical application of IONPs

The IONPs can be applied in biomedical field such as i) Targeted drug delivery ii) Hyperthermia and iii) Magnetic Resonance Imaging

2.4.1 Targeted Drug Delivery

Nanomaterials for drug delivery have two benefits namely small size and the use of biodegradable materials. The nanoparticles may extravasate through inflammatory endothelium, the epithelium (e.g., the digestive system and liver), and microcapillaries due to their small size. Generally, the nanoscale of these particles allows for efficient absorption by various cell types as well as the accumulation of drug delivery in the target area (Mou et al., 2015). Many studies have shown that the size of nanoparticles over microparticles (>1 µm) is a drug delivery method. Additionally, nanoparticles offer an advantage over bigger microparticles intravenous administration. The tiniest capillary in the body has a diameter of 5–6 µm. The particles injected into the blood must have a diameter of less than 5 µm and must not aggregate to prevent embolism (Ayubi et al., 2019).

IONPs coated with a biodegradable polymer matrix has been shown to be an effective magnetic drug carrier as a therapeutic tool. It is because, compared to uncoated IONPs, polymer-coated IONPs were found to show lower aggregation and toxicity. It is successfully used to transport small nucleic acids and therapeutic chemicals to specific organs by using EMF. The specific targeting strategy has two factors. Firstly, the IONPs exhibits magnetic properties and the altered affinity ligand on the surface of the IONPs. IONPs provide an attractive approach for precisely targeting therapeutic chemicals to disease sites. At the same time, reducing or eliminating the negative effects of cytotoxic drugs that are not absorbed by healthy tissues (Mou et al., 2015).

2.4.2 Hyperthermia

Hyperthermia is an adjuvant therapy that has been used since the turn of the century (Press, 2018). It is an anticancer therapy that raises the temperature of cancer cells by providing heat from inside the cell. Hyperthermia uses a variety of electromagnetic sources to treat cancer cells. For example, microwaves, radio waves, radiation, ultrasound, and infrared heaters. Magnetic hyperthermia uses magnetic nanoparticles created via some physical and chemical techniques. During the hyperthermia process the temperature produced in the targeted area and the duration of heat exposure. When malignant cells were treated at high temperatures, above 48 °C then more negative effects will occur. For example, protein breakdown, inactivation of cell function, and oxidative stress. The relevant temperature in the hyperthermia therapy is range from 41 to 48 °C (Kowalik et al., 2020).

2.4.3 Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is a non-invasive imaging method used to detect the structure and function of the body. It makes use of non-ionizing electromagnetic radiation that poses no danger of exposure. It uses radio frequency (RF) radiation in the presence of thorough regulated magnetic fields to create high-quality images of the body in either plane. Attaching the patient in a large magnet that produces a strong external magnetic field produces an image. The MRI has been the standard imaging method for diagnosis and prognosis. Additionally, it is an excellent biological imaging tool for monitoring therapeutic effectiveness. Moreover, cellular MRI enables the identification & monitoring of cells throughout the body. Due to the low sensitivity of this imaging method, IONPs are often employed to label the cells of interest. IONPs' magnetic properties, as well as their ability to be synthesised in a variety of sizes and forms, have led to a variety of uses. For all the remove applications, IONPs should have a high magnetization rating, a size of less than 100 nm, and a narrow size distribution (Dadfar et al., 2020).

2.5 Taguchi Method

The Taguchi method is commonly used to optimize parameter values to improve a product's quality attributes. Traditional method design is complicated, and it may not always provide the intended results. Furthermore, when the number of process parameters grows, these approaches need a high number of trials. Taguchi established the principles of Robust Design throughout the 1950s and 1960s. This technique was used in electronics, automotive, photography, and a variety of other fields. Other industries have played a significant role in the fast growth of the economy Japan's industries are growing at a rapid pace. The Taguchi method uses a unique design of orthogonal arrays that allows for a minimal number of trials to cover the whole parameter space. The advantages of the Taguchi method are it can reduce the variability of the response variable in a cost-effective manner. In addition, it can show the best way to determine the optimal process conditions during laboratory experiments. Also, it is an important tool for increasing R&D productivity, and it can be applied to any process. (Rosa et al., 2009).

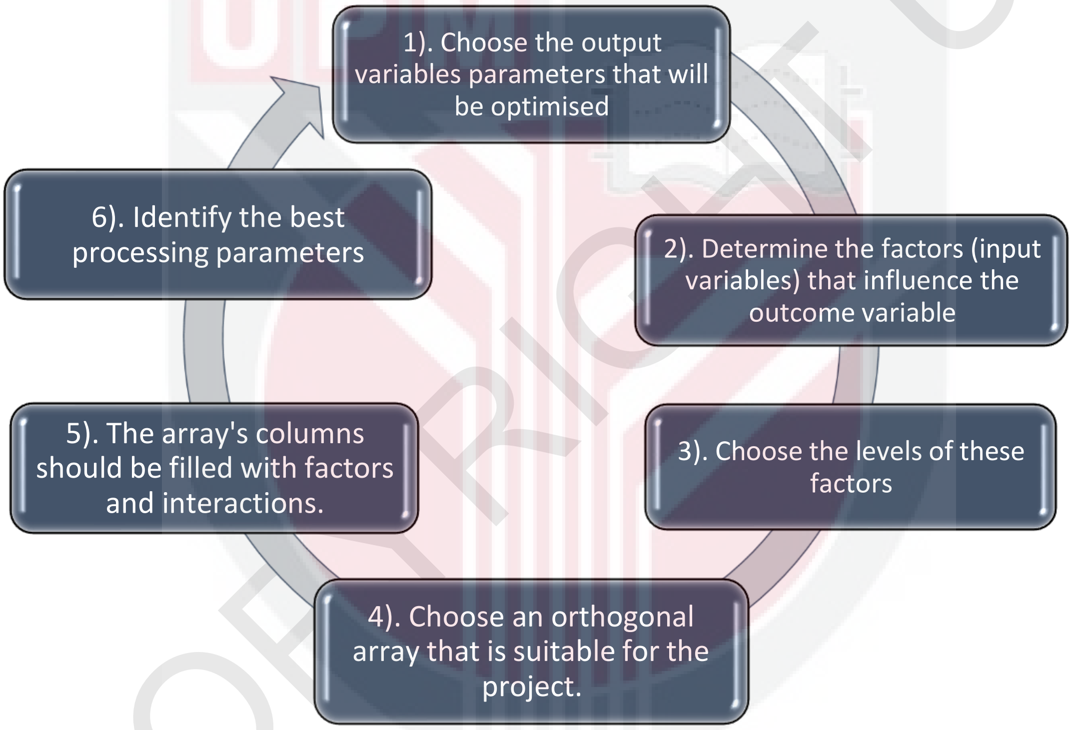


Figure 2.3: The steps of Taguchi experiment design

2.6 Conclusion

IONPs is widely used in the biomedical field, from drug delivery systems to chemotherapy drugs to imaging modalities. Recently, the green method has gained popularity since it has been shown to be a flexible strategy that may be used due of the benefits it provides. Recent improvements in the synthesis and cross-linking of monodispersed, and controllable magnetic particles. This has been allowed researches to list and overcome many of the problems related with magnetic particle therapy. IONPs is a good choice for targeted drug delivery due to their higher surface area / volume and unique magnetic properties. The potential of green IONPs in the biomedical area is in the research of their real behaviour inside complex biological systems. It will provide a comprehensive view of particle therapeutic effectiveness and toxicity

CHAPTER 3

MATERIAL AND METHODOLOGY

3.1 Materials

Roselle is gift provided by Institute of Medical Malaysia. Iron (II) chloride Tetrahydrate ($FeCl_2.4H_2O$,90 %), Iron (III) chloride Hexahydrate ($FeCl_3.6H_2O$,90 %), and Ammonium hydroxide (NH_4OH, 29 %) were obtained from R&M Chemicals to produce IONPs. The pH of the solution was adjusted using Sodium Hydroxide (NaOH, 90 %) and Hydrochloric acid (HCl, 29 %) which were bought from R&M Chemicals. Finally, acetone (C_3H_6O, 70 %) also bought from R&M Chemicals to wash the sample several times.

3.2 Methodology

The methodology in this research encompassed several methods. There is the optimization of parameter by applying Taguchi method followed up with the synthesis of IONPs using green and chemical method. Next, the characterization of IONPs and the working principle of each equipment.

3.2.1 Optimize parameter using Taguchi method

There are 3 manipulated condition; temperature (45, 65, and 85 ^{o}C), pH (8,10, and 11), volume of roselle extract (0.1,0.2, and 0.4) and rpm (600, 800, and 1000). The parameter was entered into Minitab® Software (version 19.2020.1) using the Taguchi technique of experiment design.

The Taguchi is an experimental design technique which flexible modeling that is widely used in industry. Taguchi allows value that is near to the expected value, thus increasing product quality. Taguchi is a simple tool and suitable for a wide variety of research fields. simple tool. Taguchi technique allows the study of a large number of different factors without the need for an excessive amount of testing. For instance, testing all variables in a process with eight variables, each with three states, would need 6561 (3^8) trials. However, when Taguchi's orthogonal arrays are used, just 18 trials are required, or less than 3% of the original amount. It is allows the discovery of critical factors that have the greatest impact on the performance characteristic value. Moreover, it is possible for future testing on this parameter while ignoring others that have a negligible effect (Khajeheian et al., 2018). There are 9 different parameter established using Taguchi Method as shown in Table 3.1

Table 3.1 Variable generated by Taguchi method via Minitab software

Temperature (°C)	pH	Volume (ml)	rpm
45	8	0.1	600
45	10	0.4	800
45	11	0.2	1000
65	8	0.2	800
65	10	0.1	1000
65	11	0.4	600
85	8	0.4	1000
85	10	0.2	600
85	11	0.1	800

3.2.2 Synthesis of IONPs by chemical method

In the chemical method we use co-precipitation method to synthesize IONPs. This is because it is low cost due to this method does not need much energy and pressure compared to other method. About 1.988g of Iron (II) Tetrahydrate ($FeCl_2.4H_2O$) and 5.406g of Iron Hexahydrate ($FeCl_3.6H_2O$) were mixed and dissolved in DI water in 150ml. For Taguchi optimization pH readings were taken using a pH meter based on the parameter (pH 8, 10, and 11). By adding Sodium Hydroxide (NaOH) to get more alkaline or adding hydrochloric acid (HCl) to get more acidic. Then, 20 ml of Ammonia (NH_4OH) was added into the solution as precipitating reagent. The solution was stirred by using magnetic stirrer for 1 h at rpm (600, 800, and 1000) to ensure the particle was immersed in a liquid. The mixture was bubbled with nitrogen gas to dissociate on iron solution surfaces. After 1 h a black precipitate formed in solution. The solution has been washed using acetone by centrifugation several times for 1 h at 600 rpm for 20 minutes to get a clear sample. Finally, the samples were dried in oven at 80 ^{o}C for 48 h.

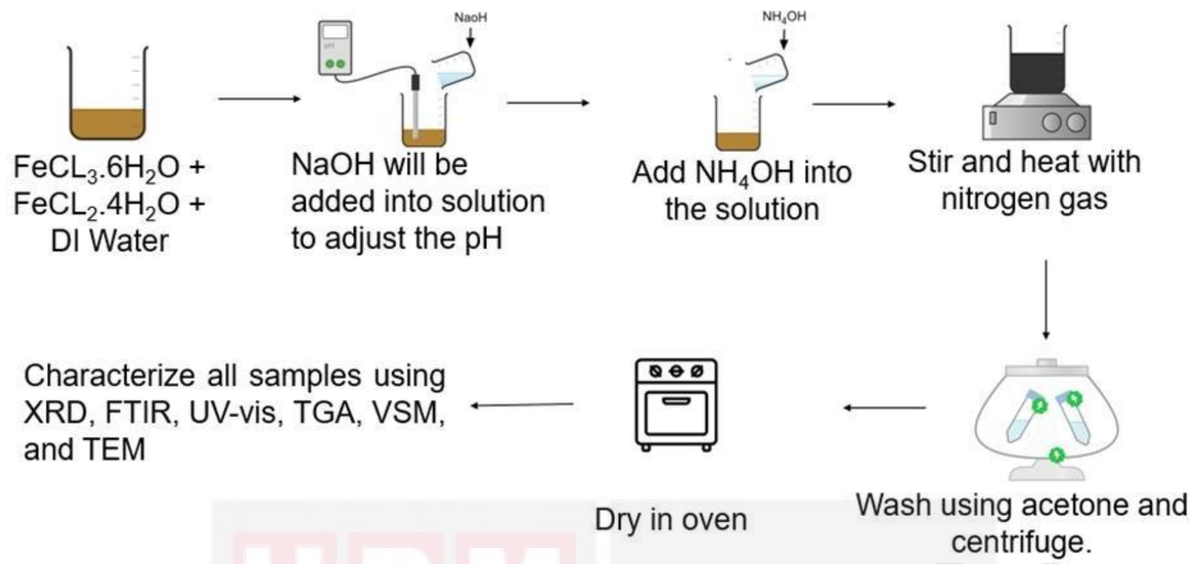


Figure 3.1: Synthesized iron oxide nanoparticles by chemical method co-precipitation

3.2.3 Preparation of plant extract

The fresh flowers will be washed using DI water to remove impurities. About 40 g of roselle flower were prepared and was mixed into with DI Water (400 ml) to form a reddish colour solution. The solution of plant extract was stirred by using hot plate magnetic stirrer at 60 ^{o}C for 2 h to ensure the roselle is immersed in liquid. After 2 h the solution will be filtered and extract stored at 4 ^{o} C to preserve humidity and prevent contamination.

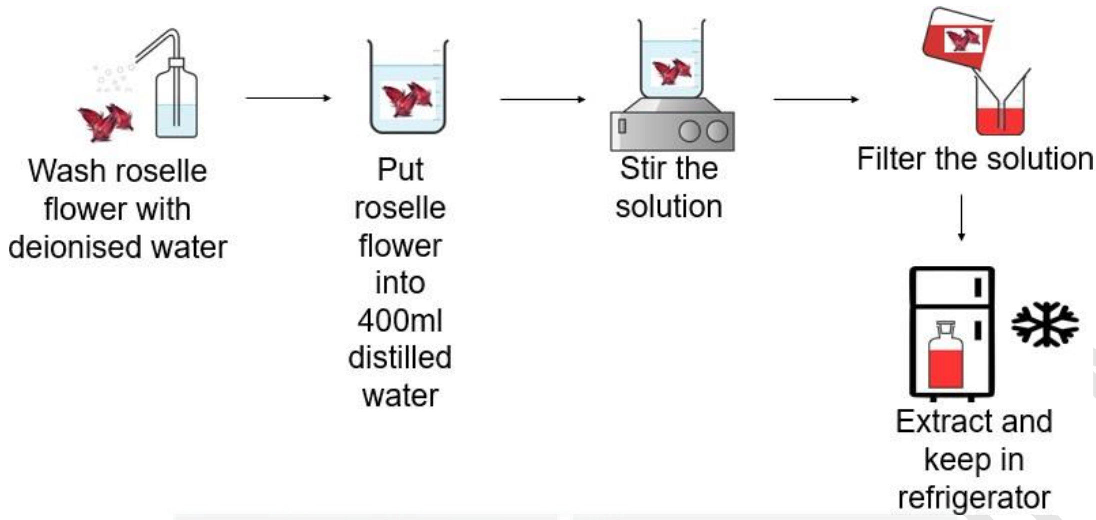


Figure 3.2: Preparation of Roselle Extract

3.2.4 Synthesis iron oxide nanoparticles by green method

About 1.988 g of Iron (II) chloride Tetrahydrate ($FeCl_2.4H_2O$) and 5.406 g of Iron (III) chloride Hexahydrate ($FeCl_3.6H_2O$) were mixed and dissolve in DI water in 150 ml. Then, plant extract (20, 40, and 80 ml) will be added and followed up by adding 20 ml of Ammonia (NH4OH) as precipitating reagent. Next, the pH is adjusted to the specified pH describe in Taguchi method (8, 10, and 11). The solution was stirred using magnetic stir for 1 h at the specified rpm (600, 800, and 1000 rpm). This to ensure the particle was immersed in a liquid and will be purged with nitrogen gas to dissociate on iron solution surfaces. After 1 h, a black precipitate formed in solution and washed by using acetone via centrifugation for several times. Finally, it is dried in oven at 80 ^{o}C for 48 h.

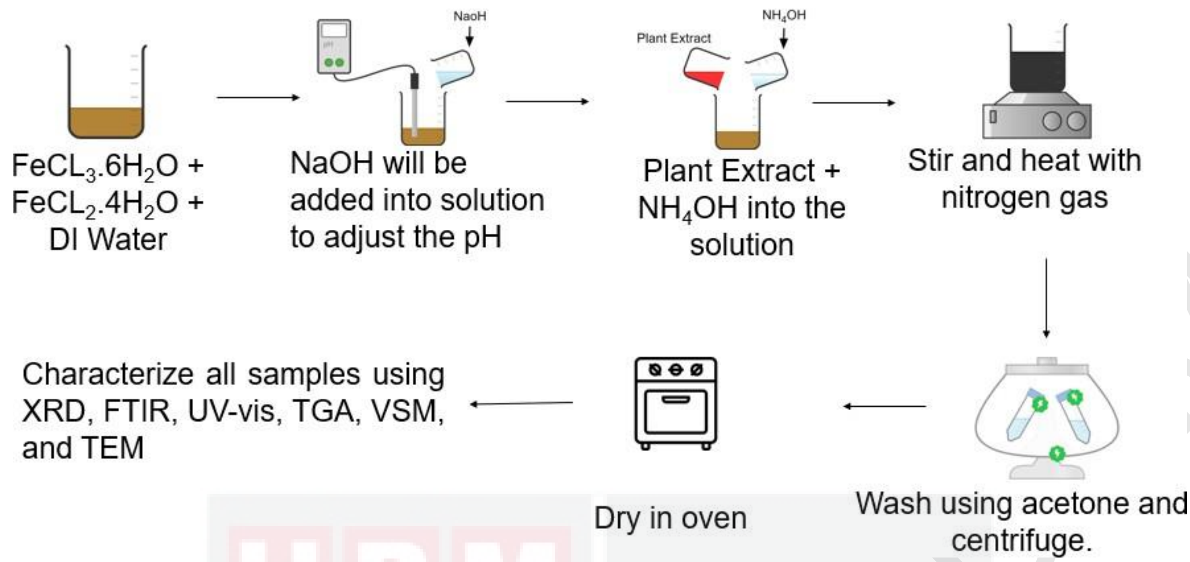


Figure 3.3: Synthesized of iron oxide nanoparticles by roselle flower

3.3 Characterization of IONPs

IONPs will be characterized by various of techniques to determine absorption, structural, magnetic, functional group, thermal stability and its surface morphology. The absorption and structural properties were investigated by using UV-Vis Spectroscopy (UV-Vis) and X-Ray Diffraction (XRD) respectively. Additionally, the magnetic behaviour and its functional groups were identified via Vibrating Sample Magnetometer (VSM) and Fourier Transform Infrared (FT-IR) correspondingly. The thermal stability and its surface morphology were defined by using Thermal Stability Analysis (TGA) and Transmission Electron Microscopy (TEM) consecutively

3.3.1 UV-Vis Spectroscopy

UV-Vis Spectroscopy (UV-Vis) is a kind of spectroscopy that involves measuring the attenuation (weakening of the strength/intensity) of a beam of light after it passes through or reflects off a sample surface. UV-Vis spectroscopy is used to determine the absorbance spectrum of a material in solution or as a solid.

UV-vis spectroscopy is a kind of molecular spectroscopy that follows a Lambert Beer law. Electromagnetic waves are used to measure the Surface plasmon and total electron oscillations in the band gap. It may also be used to monitor fluid and other chemical absorption. UV-visible spectroscopy divides a light beam in two parts. One half investigating the chemical or solutions inside the transparent cell. The other half studying the components of the reference material. This research was supported by the fact that the solution absorbs light at a specific wavelength, known as the chemical surface plasmon resonance (SPR).

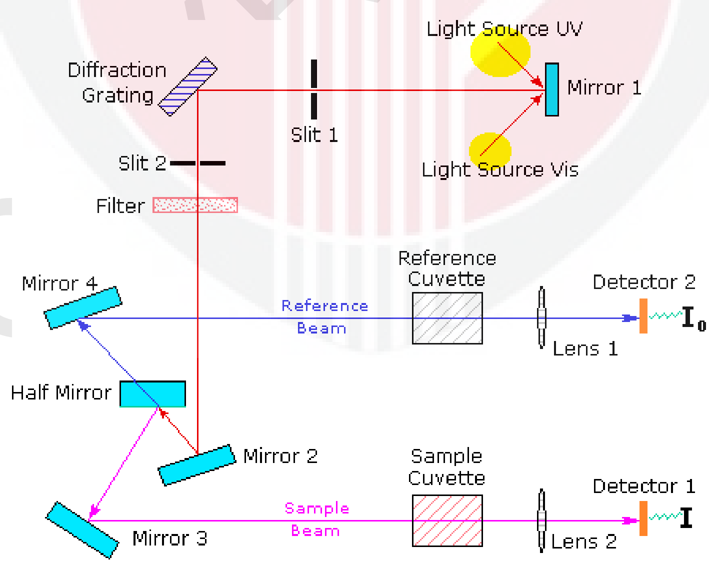


Figure 3.4: UV-Vis spectroscopy (Source : http://goo.gl/Pg9X85)

3.3.2 Fourier Transform Infrared

Fourier Transform Infrared (FT-IR) analysis allows the determination of chemical bonding in a sample by analysing the IR spectra absorbed by a particular substance. When infrared light is absorbed by the sample's molecules, they may be excited to a higher energy level. Infrared radiation has an energy range that corresponds to a stretching and bending natural frequency of the bonds in the majority of covalent compounds. The frequency at which chemical bonds vibrate is governed by their composition, bond angle, and lengths. By absorption certain wavelengths of light, individual molecules may interact with incoming radiation.

Absorption occurs when FTIR light interacts with the vibration frequency of a bond that has the same frequencies at a particular resonance frequency. The form of molecular energy, the vibronic coupling strength, and the atomic mass all have an effect on the absorption of a specific energy. The variation observed in the FTIR spectrum of various chemicals or molecules is the result of the unique configuration of the atoms. The absorbed energy produces bond amplitudes in the molecules' vibrational movements. Infrared light with frequency that correspond to the inherent vibrational movements of chemical molecules. Absorption is possible for infrared radiation with frequency that match the natural molecule vibrational frequencies. However, not all connections absorb infrared light. Infrared light can be absorbed exclusively via dipole-moment connections. Infrared light cannot be absorbed by symmetrical bonds. Additionally, FTIR may be utilised to obtain information about the structure of a molecule.

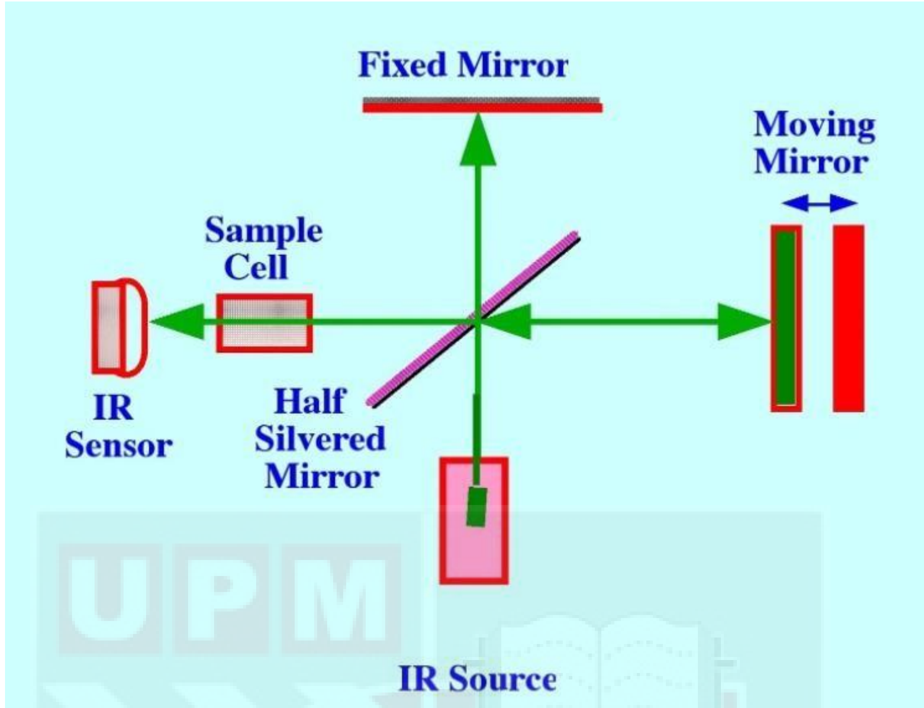


Figure 3.5: The standard structure of FT-IR Spectroscopy (Source : https://aavos.eu/glossary/ftir/)

CHAPTER 4

RESULT AND DISCUSSION

4.1 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis was used to find the presence of biomolecules in red petal from roselle extract that were responsible for bio-reduction and stabilization of IONPs. The production of IONPs process is through the reduction of iron metal ions. The synthesised IONPs were analysed by FTIR in order to determine which functional groups were present in the sample. In figure 4.1, 4.2 and 4.3 shows that the peak intensities in transmittance for the wavenumber range 400 - 4000 cm^{-1} spectrum region was analysed and shown. In addition, a broad peak at 3191, 3203, 3184, 3216, 3188, 3204 and 3197 cm^{-1} for each graph is assigned to O-H stretching frequency indicating the presence of hydroxyl groups. The peak at 1600 cm^{-1} is mainly indicating of the possible C=C aromatic and 1125 cm^{-1} for C–O–C bonds. The band 525, 514, 550, 521, 526, 571, 529, 514 and 573 cm^{-1} present the Fe-O stretching vibrational group. As a result of the FTIR study, the multifunctionality of roselle extract is revealed, with simultaneous reduction and stabilization.

These FTIR peaks correspond to previously published work on synthesised IONPs by extract of hibiscus flower extract (Alshehri et al., 2017). According to Alshehri et al., (2017), the FTIR spectrum shows a peak at 1629 cm^{-1} for C=C. Peak shifts between 514 cm^{-1} until 638 cm^{-1} indicate the importance of the Fe-O stretching vibrational group. The peak at 1629 cm^{-1} is mainly indicating of the possible C=C aromatic and 1066 cm^{-1} for C–O–C bonds.

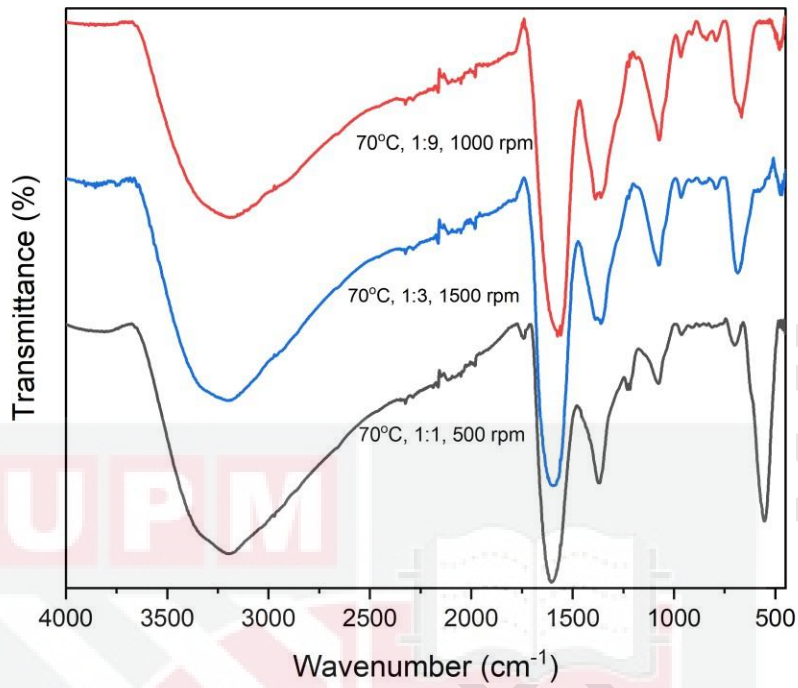


Figure 4.1: FTIR spectra of IONPs at 70°C

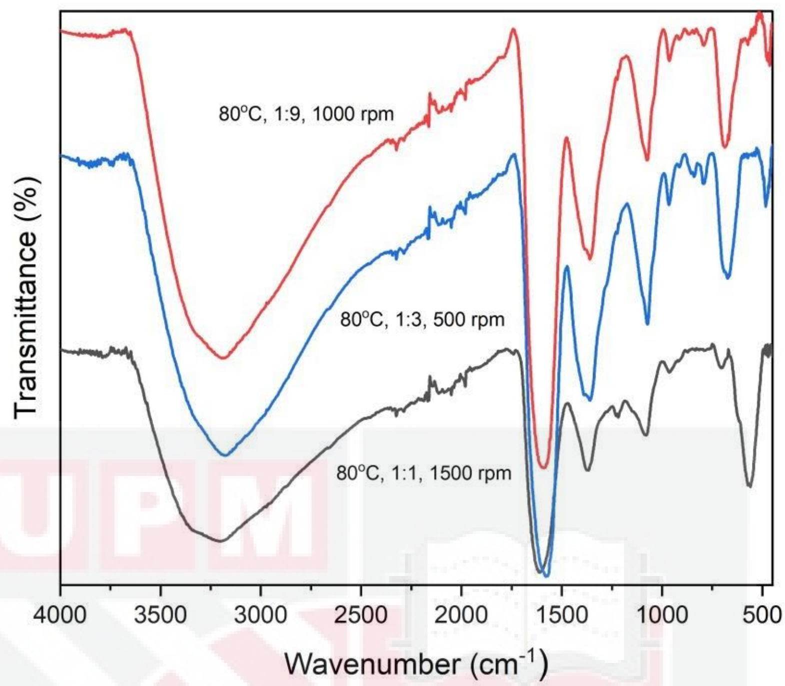


Figure 4.2: FTIR spectra of IONPs at 80°C

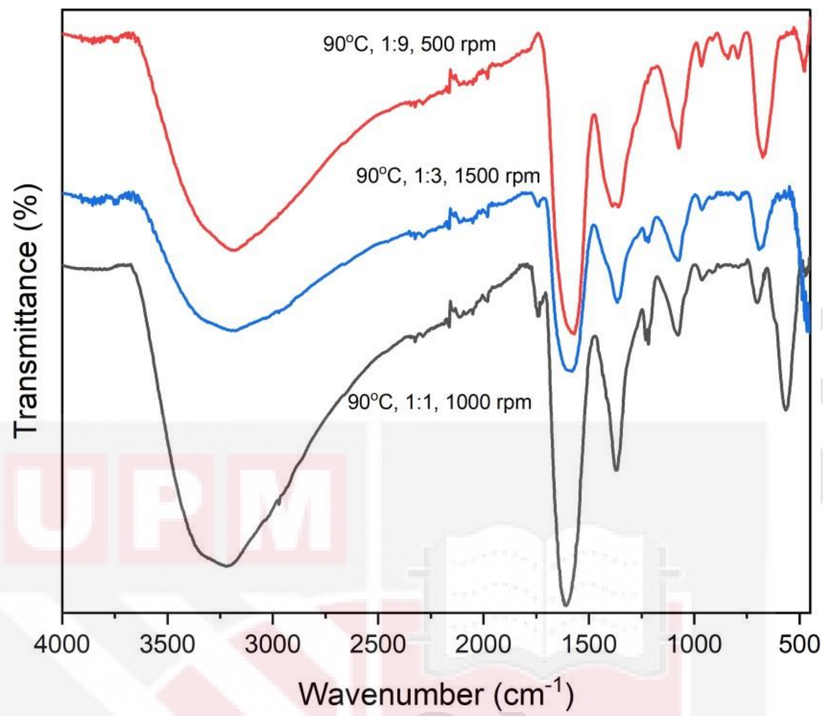


Figure 4.3: FTIR spectra of IONPs at 90°C

4.2 Ultraviolet-Visible Spectroscopy (UV-Vis)

UV–vis absorption spectroscopy is a technique to determine the optical properties of IONPs. UV-visible spectroscopy is a useful method for studying the kinetics of Fe nanoparticle formation. The beginning of the reduction process iron ion (Fe^{3+}) to iron atom (Fe^{0}) and the formation of IONPs was confirmed by this consecutive colour change to a brown colour when the roselle red extract was added with $FeCl_3$ and $FeCl_2$. In figure 4.4, 4.5 and 4.6 shown for 70°C that the synthesis IONPs by roselle flower has two peaks 210 nm, 211 nm, 219 nm and 260 nm. Then. for 80°C shown two peaks 210 nm, 211 nm, 213 nm, 259 nm and 260 nm. Nextly, for 90°C shown that has 211 nm, 212 nm, 259 nm and 260 nm. The pure roselle extract peaks at above peaks correspond due to the extract's presence of a variety of natural moieties. Fe nanoparticles synthesised by roselle flower extract showed a strong absorption peak at 259 and 260 nm. This suggests that several components in the extract worked as reducing and capping agents during the Fe nanoparticle production. The hydroxyl groups in biologically active molecules bind to Fe^{3+} to form ferric hydroxide, which is then partially reduced by other bioactive materials to form Fe nanoparticles.

These results were similar to the results from research conducted by (Aksu Demirezen et al., 2019). Aksu Demirezen et al., (2019) state that with time, the intensity of the peak increases. IONPs have a UV absorption peak range between 205 and 291 nm, which is mostly owing to surface plasmon resonance (SPR). The SPR band was produced when free electrons in IONPs were excited while they were absorbing visible light.

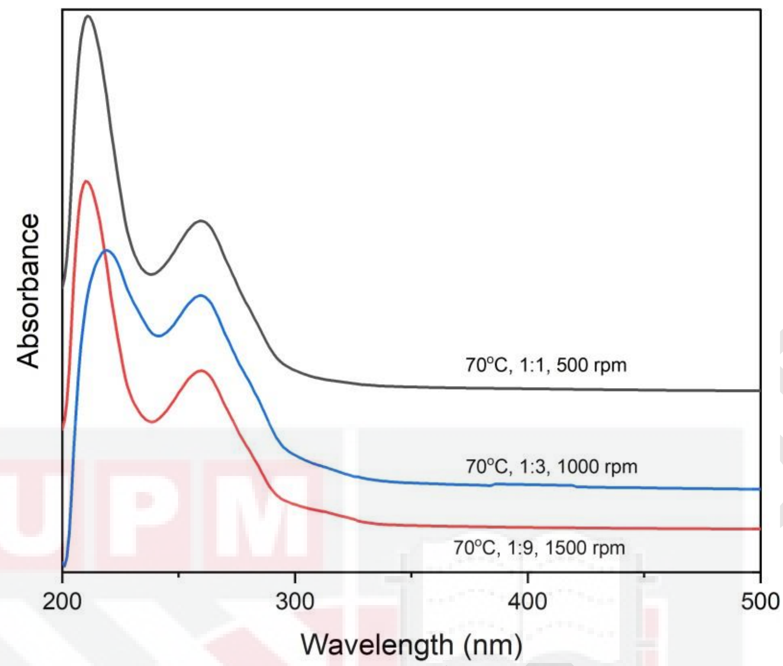


Figure 4.4: UV-Visible spectra of IONPs at 70ºC

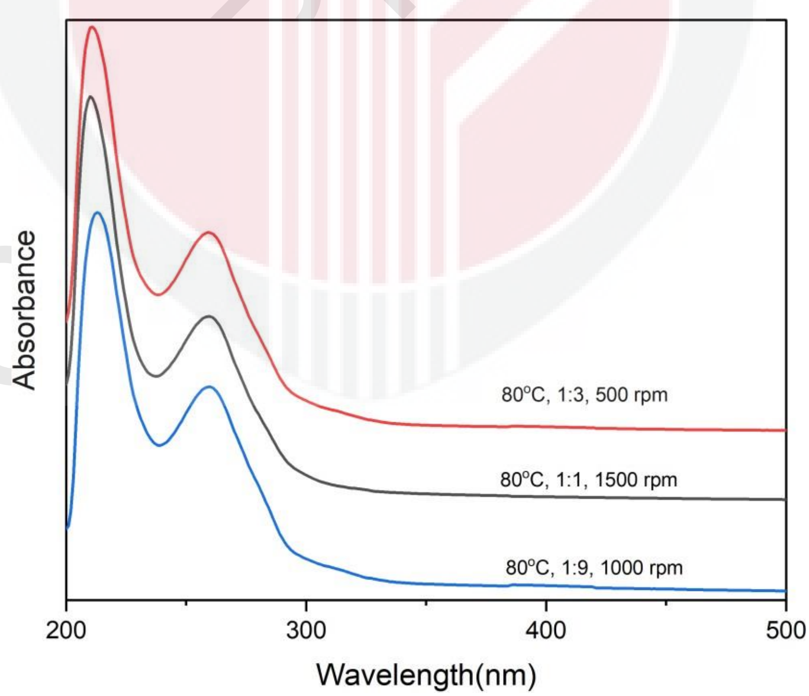


Figure 4.5: UV-Visible spectra of IONPs at 80ºC

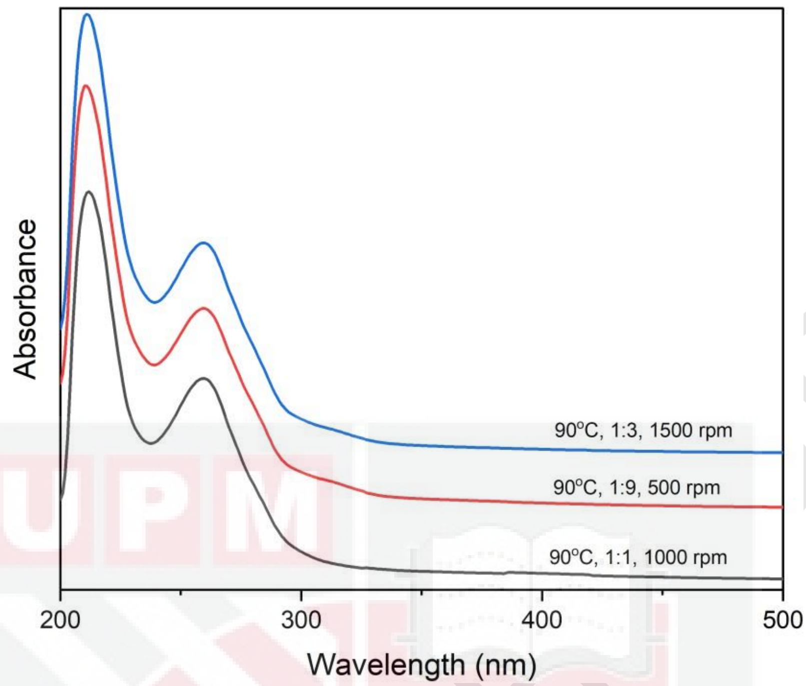


Figure 4.6: UV-Visible spectra of IONPs at 90ºC

CHAPTER 5

CONCLUSION

5.1 Conclusion

In this study, IONPs have been successfully synthesised using a green method that included Roselle extract as a new reducing and stabilizing agent. The volume of Roselle extract used to synthesis IONPs was successfully optimized. UV-Vis was used in this research to determine the optical properties of IONPs. Green synthesized IONPs were prepared using different volumes of Roselle extract. From UV-Vis analysis, a plasmon resonance band was detected between 210, 211, 213, 219 nm and 259, 260 nm. The wavelength is increased because the rate of absorption. For IONPs, the FTIR results suggest the presence of biomolecules in red petal from roselle extract, a broad peak at 3188 until 3204 cm^{-1} for each graph is assigned to O-H stretching frequency indicating the presence of hydroxyl groups. The peak at 1600 cm^{-1} is mainly indicating of the possible C=C aromatic and 1125 cm^{-1} for C–O–C bonds. The band 525, 514, 550, 521, 526, 571, 529, 514 and 573 cm-1 present the Fe-O stretching vibrational group

5.2 Recommendations

Due to COVID-19 in Malaysia, some of the characterizations in the ongoing investigation were difficult to complete. IONPs will be investigated in the future by using Transmission Electron Microscopy (TEM), Thermogravimetric Analysis (TGA), X-ray Diffraction (XRD) and Vibrating Sample Magnetometer (VSM). Additionally, IONPs can be synthesis by using green methods other than green co-precipitation method like green so-gel, green hydrothermal and green thermal decomposition. This aims to make comparison in terms of experimental results among the types of methods between experimental results. Therefore, the green synthesis methods of IONPs must be improved to create a product that can be produced in large quantities with sufficient space for future characterization and deployment. Moreover, IONPs can be used in a variety of application like biomedical application, storage energy, electronics and animal bio. The application of IONPs should be improved in future studies. After that, the next suggestion is to evaluate influencing parameters like time, pH, concentration and temperature. Lastly, the IONPs can be synthesized by using other than roselle flower. Also, IONPs can be synthesized by plant extract by using other part of plant such as root, leaf and branch.

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Course	Bachelor Science (Hons)
Major	Science Instrumentation
Permanent Address	No.401 Lorong KTC 4/11, Ktc Golf View, 09000, Kulim, Kedah Darul Aman.
Phone Number	: +601155094908
Email Address	198134@student.upm.edu.my
Education Background	. Sek. Keb. Taman Hi-Tech Sek. Men. Keb Taman Hi-Tech Aliran Fizik, Kolej Matrikulasi Kedah, Changlun, Kedah Universiti Putra Malaysia.