COLORIMETRIC DETECTION OF HEAVY METAL IONS USING SILVER NANOPARTICLES SYNTHESIZED FROM *Alpinia galanga*

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by

MUHAMAD ZULHELMI BIN NOR ARZUAN

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LIST OF ABBREVIATIONS

AgNPs	Silver Nanoparticles
LPSR	Localized Plasmon Resonance
$AgNO_3$	Silver Nitrate
SEM	Scanning Electron Microscopy
DLS	Dynamic Light Scattering
EDX	Energy Dispersive X-Ray
USEPA	United State Environmental Protection Agency
SPR	Surface Plasmon Resonance
NADH	Nicotinamide Adenine Dinucleotide Hydrogen
TPC	Total Phenolic Content
TFC	Total Flavonoids Content
CBPE	*Cinnamon Zeylanicum* Bark Extract

PENGESANAN KOLORIMETRI ION-ION LOGAM BERAT MENGGUNAKAN NANOPARTIKEL PERAK YANG DISINTESIS DARIPADA *Alpinia Galanga*

ABSTRAK

Nanopartikel logam seperti emas (Au), perak (Ag) dan kuprum (Cu) telah digunakan secara meluas sebagai bahan penderiaan untuk pengesanan kolorimetrik ion logam dalam larutan kerana ia mempamerkan sifat resonans plasmon setempat (LPSR). Dalam kajian ini, nanopartikel perak (AgNPs) telah disintesis daripada ekstrak tumbuhan *Alpinia galanga* dengan menggunakan kaedah biologi dan sifat penderiaan kolorimetrik telah disiasat dengan menggunakan pelbagai jenis logam berat pada kepekatan 1 mM. Sintesis biologi AgNPs boleh dianggap sebagai alternatif yang lebih menjanjikan daripada kaedah kimia kerana kaedah ini lebih fleksibel, mesra alam dan kos efektif. Proses pengoptimuman dijalankan untuk mengkaji kesan kepekatan ekstrak tumbuhan, nisbah ekstrak tumbuhan kepada larutan $AgNO_3$ dan nilai pH terhadap pembentukan dan pertumbuhan nanopartikel perak. Keadaan optimum untuk sintesis AgNPs ialah 10 wt% kepekatan ekstrak tumbuhan, 1:5 nisbah ekstrak tumbuhan kepada larutan $AgNO_3$ dan nilai pH menghampiri neutral iaitu pH 7. Pengesanan kolorimetrik logam berat dan ujian sensitiviti kemudiannya dijalankan untuk tentukan keupayaan nanopartikel perak untuk mengesan ion logam dalam kepekatan kecil. Daripada pengesanan kolorimetrik logam berat, dapat diperhatikan bahawa $CuCl_2$ mempunyai perbezaan tertinggi antara puncak penyerapan AgNPs sebelum dan selepas penambahan logam berat pada 0.45475, yang juga menunjukkan bahawa AgNPs menunjukkan kepekaan yang lebih besar terhadap larutan $CuCl_2$. Keputusan daripada analisis UV-Vis dalam ujian sensitiviti menunjukkan bahawa

apabila kepekatan larutan logam berat berkurangan daripada 0.8 mM kepada 0.2 mM, puncak penyerapan akan meningkat. AgNP yang telah disediakan telah dikaji ciri-ciri dan sifatnya dengan menggunakan spektrofotometri UV-Vis, potensi zeta, pengimbasan mikroskop elektron (SEM) dan analisis sinar-x penyebaran tenaga (EDX). Analisis EDX mengesahkan kehadiran zarah perak pada kadar berat 61.21% manakala morfologi AgNP ditentukan oleh analisis SEM. Nanopartikel didapati mempunyai morfologi hampir sfera daripada imej SEM. Daripada analisis potensi zeta, nanopartikel terbukti mempunyai sistem koloid yang stabil kerana ia mempunyai nilai potensi zeta negatif yang besar pada -39.3 mV.

COLORIMETRIC DETECTION OF HEAVY METAL IONS USING SILVER NANOPARTICLES SYNTHESIZED FROM *Alpinia Galanga*

ABSTRACT

The metal nanoparticles such as gold (Au), silver (Ag) and copper (Cu) have been extensively utilized as a sensing material for colorimetric detection of metal ions in a solution since they exhibit localized plasmon resonance (LPSR) properties. In the present study, silver nanoparticles (AgNPs) were synthesized from *Alpinia galanga* plant extract by using biological method and colorimetric sensing properties were investigated by using various type of heavy metal at concentration of 1 mM. Biological synthesis of AgNPs can be considered as more promising alternatives than chemical methods because it is more flexible, environmentally friendly and cost-effective method. Optimization process was conducted to study the effect of plant extract concentration, ratio of plant extract to $AgNO_3$ solution and pH value on the formation and growth of AgNPs. The optimal condition for AgNPs synthesis is plant extract concentration of 10 wt%, ratio of plant extract to $AgNO_3$ solution of 1:5 and pH value near to neutral which is pH 7. Colorimetric detection of heavy metal and sensitivity test then were carried out to determine the ability of AgNPs to detect metal ions in small concentration. From heavy metal colorimetric detection, it can be observed that $CuCl_2$ has the highest difference between the absorbance peak of AgNPs before and after the addition of heavy metals at 0.45475, which also indicates that AgNPs show greater sensitivity towards $CuCl_2$ solution. The result from UV-Vis analysis in the sensitivity test demonstrated that when the concentration of heavy metal solution decreases from 0.8 mM to 0.2 mM, the absorbance peak will increase. The prepared AgNPs was characterized by using UV-Vis spectrophotometry, zeta potential,

scanning electron microscopy (SEM) and energy dispersive x-ray (EDX) analysis. The EDX analysis confirmed the presence of silver particles at 61.21 % of weight whereas the morphology of AgNPs were determined by SEM analysis. The nanoparticles were found to have almost spherical morphology from the SEM images. From zeta potential analysis, the nanoparticles proved to have stable colloidal system as it has large negative zeta potential at -39.3 mV.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Pollution of heavy metal has become a severe threat to the environment especially to human health for many years. Heavy metals are non-biodegradable material that are ubiquitously distributed and can be considered as low density chemical components that are highly toxic (Gumpu *et al.*, 2015). The heavy metal's toxicity depends on their concentration and their speciation. These components can be toxic and can alters the life cycle of biochemical when they enter the cell because of the bond formation of metals with thiol group of proteins. It can cause greater risk to human health once it enters living organisms since they get accumulated in the biosphere. These toxic heavy metals such as cadmium chloride, copper chloride, etc. also tends to bioaccumulate in polluted ground water and soil as they move up the food chain.

Heavy metal refers to any metallic or metalloids element that has relatively high density (with atomic density greater than 4 g/cm^3) and it is toxic even at low concentration. Heavy metals include lead (Pb), cadmium (Cd), zinc (Zn), mercury (Hg), arsenic (As), silver (Ag), chromium (Cr), copper (Cu), iron (Fe) and platinum group elements. Heavy metal can be emitted into the environment by both natural and anthropogenic causes. It is proven that anthropogenic causes such as mineral sources development, metal processing and smelting, chemical production, factory emission and sewage irrigation are the primary causes of heavy metal pollution. Unlike organic pollutant, heavy metal pollutant cannot be biodegraded once it introduced into the environment. Thus, the emission of heavy metal needs to be monitored by the authorities so that there is no excess emission of heavy metal that can leads to pollution

especially at industrial area such as mining area. Table 1.1 shows maximum contamination levels for heavy metal concentration in air, soil and water by United State Environmental Protection Agency (USEPA).

Table 1.1 United State Environmental Protection Agency maximum contamination levels for heavy metal concentration in air, soil and water

Heavy metal	**Max conc. in air (mg/m^3)**	**Max. conc. in sludge (soil) (mg/Kg or ppm)**	**Max. conc. in drinking water (mg/l)**	**Max. conc. in H_2O supporting aquatic life (mg/l or ppm)**
Cd	0.1-0.2	85	0.005	0.008^{δ}
Pb	-	420	0.01" (0.0)	0.0058^{δ}
Zn^2	1, 5*	7500	5.00	0.0766^{δ}
Hg	-	<1	0.002	0.05
Ca	5	Tolerable	50	Tolerable >50
Ag	0.01	-	0.0	0.1
As	-	-	0.01	-

The development of silver nanoparticles (AgNPs) increases significantly in recent year since they have potential application in various fields including chemistry, biological and medical. AgNPs has proven as a good detector for various chemicals through colorimetric sensing due to its effectives optical features called surface plasmon resonance (SPR). Therefore, AgNPS has received huge attention for detection of heavy metal because it offers simple operation procedure without using expensive equipment. In this project, AgNPs were synthesized using *Alpinia galanga* extract as the reducing and capping agent. The characterization of AgNPs were conducted using UV-vis spectrophotometer, scanning electron microscopy (SEM), zeta potential and energy disperse x-ray (EDX) while the potential of AgNPs colorimetric sensing will be tested through monitoring the color changes by naked eyes and UV-vis spectrophotometer.

1.2 Problem Statement

Based on the literature review, chemical method extensively used as a method to synthesis AgNPs because of its short reaction time (Joseph and Mathew, 2014). However, we know that the challenges faced when using chemical method is that chemical method utilize toxic chemical such as sodium citrate as the reducing and capping agents. Toxic chemicals are not environmentally friendly and can cause severe environmental pollution. Moreover, it requires harsh reaction condition. In this research, the synthesis of AgNPs by using *A. galanga* extract has been proposed as a remedy to this problem. Biological synthesis of AgNPs is a more promising alternatives than chemical methods because it is more flexible, environmentally friendly, has shorter reaction time and require lower energy consumption. Several parameters were studied on the formation of AgNPs using *A. galanga* such as the concentration of plant extract, ratio of *A. galanga* extract to $AgNO_3$ and pH value to understand the influence of these parameters to synthesis AgNPs. Whereas the characterization of AgNPs will be observed using UV-vis spectrophotometer, SEM, zeta potential and EDX analysis. Besides, the heavy metal colorimetric sensing properties of the synthesized AgNPs from *A. galanga* plant extract was also studied.

1.3 Research Objectives

The objectives of this research are:

i. To determine the effect of plant extract concentration, ratio of plant extract to $AgNO_3$ solution and the pH value to the biosynthesis of AgNPs

ii. To characterize the synthesized AgNPs

iii. To evaluate the colorimetric sensing potential of AgNPs synthesized by *A. galanga* plant extract towards heavy metal ions in aqueous solution

CHAPTER 2

LITERATURE REVIEW

2.1 Silver Nanoparticles

Nanoparticles exhibit superior physical and chemical properties due to an increase in the ratio of the surface area per unit volume compared to its bulk structure. Metal nanoparticles (silver, gold, etc.) are the most studied nanoparticles since these materials are easier to produce with a size range between 1 to 100 nm. Due to their unique physical and chemical properties, AgNPs are increasingly applied in a broad field such as health care, medical and industrial purpose. It also has been used as antibacterial agents, optical sensors, medical device coatings, health-care-related products, in the food industry and the pharmaceutical industry since they have distinct physical and optical properties, as well as biochemical functionality tailored by a variety of size- and shape-controlled AgNPs.

Silver or Argentum (Ag) is a chemical element with the atomic number 47. Chemically, silver is the transition element of group 11 element. The oxidation number of Ag varied from -2 to +3 but mostly Ag^+ dominates its chemistry. Due to their distinct photoelectric and physiochemical properties, this noble metal has gotten great attention in recent years with the progress of nanoscience.

2.2 Silver Nanoparticles Synthesis

Silver nanoparticles can be synthesized via different chemical and physical methods, namely chemical reduction, thermal decomposition, laser ablation and also biological method. Compared to the biological synthesis method which has appealing properties, the physical and chemical synthesis method tend to be more hazardous and labor-intensive (Zhang *et al.*, 2016). Each of the listed methods has advantages and

disadvantages and morphologies and characterization of silver nanoparticles depends on its characterization tools.

2.2.1 Physical Method

Evaporation-condensation and laser ablation are the most important physical approaches to synthesize AgNPs. In evaporation-condensation, metal nanoparticles are typically synthesized by using a tube furnace at atmospheric pressure. The source material namely, Ag, gold (Au), phosphate-buffered saline (PS) and fullerene within a boat which located at center of the furnace is vaporized into a carrier gas (Dheeksha, 2018). Figure 2.1 illustrated the synthesis of AgNPs by the evaporation-condensation method. Dheeksha (2018) demonstrated that the geometric mean diameter of AgNPs synthesized through this method was in a range between 6.2 nm to 21.5 nm. Laser ablation is a method to fabricate the various type of nanoparticles by nucleation and growth of laser vaporized species with a background gas. It also includes semiconductor quantum dots, carbon nanotubes, nanowires and core shells nanoparticles. High purity nanoparticles can be generated by this method since the AgNPs purity is dependent on the purity of the target and ambient media (gas or liquid) without contamination from the reactor (Suriati, Mariatti and Azizan, 2014). The schematic particle generation procedure in the laser ablation process is illustrated in Figure 2.2.

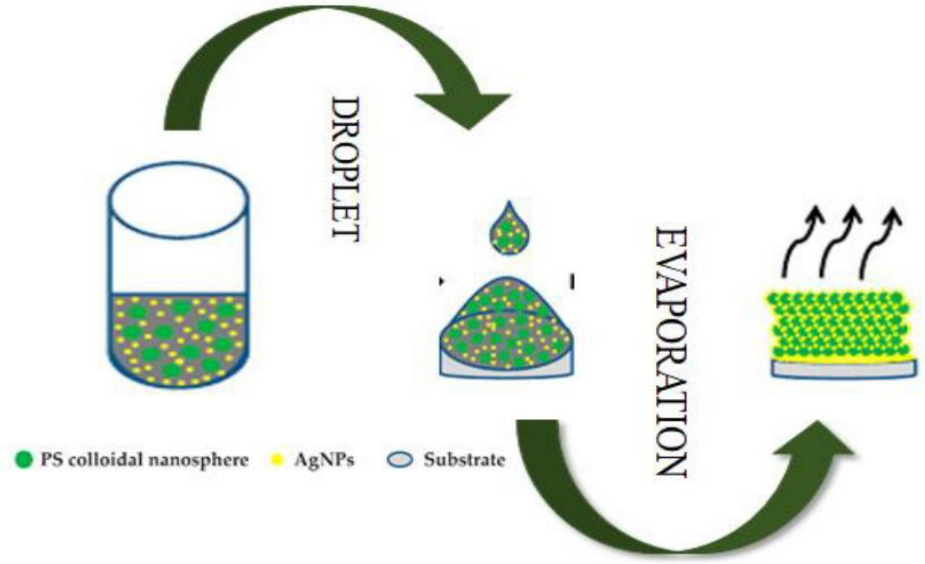

Figure 2.1 Synthesis of silver nanoparticles by evaporation-condensation method (Dheeksha, 2018)

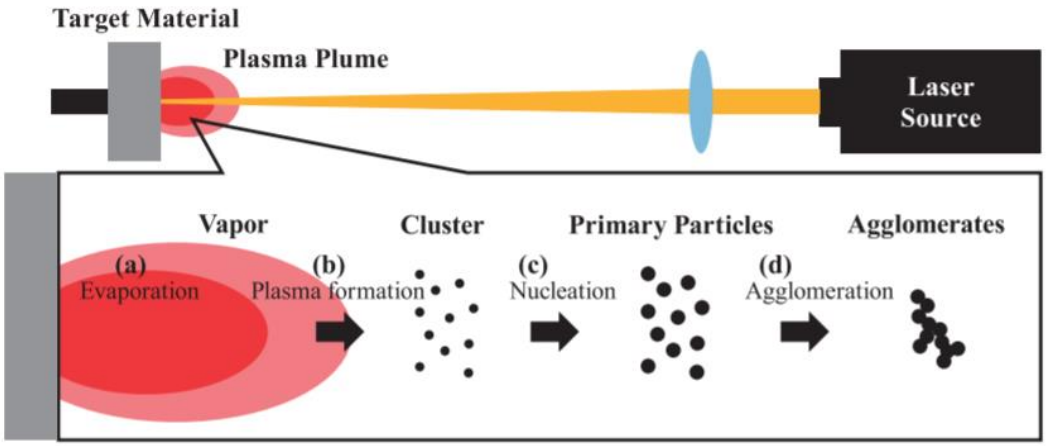

Figure 2.2 The schematic of the particle generation procedure in laser ablation process (Suriati, Mariatti and Azizan, 2014)

Other physical approaches such as high energy ball milling (HEBM) and arc-discharge also can be used to synthesize AgNPs, however, this method is not popular in the industry since it is operated in harsh conditions. Studies reported that the HEBM method is operated at a temperature greater than 1000 °C and its operating pressure can be increased up to several GPa (Prabakaran and Rajan, 2021). In this process, small nanoparticles are formed by the hard collision between the bulk material and kinetic energy of the balls which results in a breakdown of chemical bonds in bulk material and

generates a new surface. The arc discharge is another physical method that utilized electric discharge between silver electrodes under the effect of a strong electric field with high temperature. Under this condition, plasma is generated and caused silver atoms to be removed from the electrode's surface. Water dissociates into an oxygen atom and hydrogen atoms in an aqueous solution. The interaction occurred between active silver atoms with oxygen and hydrogen atoms results in the formation of a stable nanoparticle colloid (Jabłońska *et al.*, 2021). This physical method does not utilize any toxic chemical, typically has a fast-processing time, radiation is used as a reducing agent and AgNPs formed has narrow size distribution (Asanithi, Chaiyakun and Limsuwan, 2012). However, this method is associated with major drawbacks which are low yield and high energy consumption, solvent contamination and lack of uniform distribution (Zhang *et al.*, 2016).

2.2.2 Chemical Method

In most cases, chemical methods utilized toxic chemicals especially organic solvents as a stabilizer and reducing agent. These chemicals can lead to a variety of health and environmental problem. In addition, a long reaction time is required for chemical methods to complete the reduction process at room temperature (Parida, Bindhani and Nayak, 2011). Chemical reduction by organic and inorganic reducing agents is the most common chemical approach for the synthesis of AgNPs. Sodium citrate, sodium borohydride, elemental hydrogen, polyol process and poly (ethylene glycol)-block copolymers are an example of different type of reducing agent that has been utilized to reduce silver ions (Ag^{+}) in aqueous and non-aqueous solution. It is important to select a suitable reducing agent since the size, shape and particles size distribution of AgNPs is strongly depends on the nature of the reducing agent. During this method, the reducing agent will reduce silver ions to produced metallic silver atoms.

The generated silver ions act as a nucleation center for nanoparticles and catalyzed the reduction process of the remaining metal ions present in the solution. The nuclei will grow continuously and the formation of AgNPs occurs (Suriati, Mariatti and Azizan, 2014). The change in colour of the solution indicates the formation of AgNPs and can be observed with naked eyes. To avoid agglomeration, it is crucial to use a protective agent to stabilize dispersive nanoparticles during the course of AgNPs preparation. The chemical reduction method has been applied in the industry due to its simplicity and results in high yield.

The other chemical method that can be used to generate AgNPs is microwave-assisted synthesis. This is a promising method since it offers several benefits such as shorter reaction times, better product yields which can avoid the agglomeration of the particles formed and reduced the consumption of energy (Nadagouda, Speth and Varma, 2011). According to Pal, Shah and Devi (2009), polar molecules that have been used as reducing agents try to orient with the electric field with a frequency range between 300 MHz to 300 GHz. The molecules will lose their energy in form of heat via molecular friction, when they try to orient the alternating electric field. This will result in high dielectric losses for the molecules. High dielectric losses will increase the reducing ability which is the crucial aspect to be selected as ideal solvents for microwave heating. It is reported that water and ethanol have high dielectric and high reducing ability. At the boiling point of ethanol, the dielectric loss constant for ethanol is 6.08. In this method, the state of silver cations in the initial reaction solution is strongly affected by the size distribution of the synthesized AgNPs. Although this chemical approach has various advantages for the synthesis of AgNPs, this approach has been associated with certain drawbacks including its utilization of toxic chemicals that can lead to several health problems.

2.2.3 Biological Method

To overcome the drawbacks of chemical methods, biological methods are the best alternatives since it straightforward, cost-effective and environmentally friendly (Jie, Nadzir and Rangasamy, 2020). This biological approach offers a potential alternative to chemically synthesized AgNPs since it incorporates the use of biological agents such as fungi and plant extract as reducing and capping agents. Same as chemical and physical methods, the size and morphologies of synthesized AgNPs are highly dependent on some critical aspects, including types of organism, properties of the organisms, optimal reaction conditions and selection of biocatalyst state. Table 2.1 illustrates the example of organisms used for synthesizing AgNPs.

Table 2.1 The example of organism used for synthesizing AgNPs (Zhang *et al.*, 2016)

Biological synthesis of silver NPs			
Bacteria	**Plant**	**Fungi**	**Algae**
Aeromonas sp. SH10 (Bacterium)	*Aloe vera* leaf extract (Plant)	Nitrate reductases (from *Fusarium oxysporum*)	*Spirulina platensis* (Alga)
Klebsiella pneumonia (Bacterium)	*Azadirachta indica* (Plant)	*Phaeneroechaete chrysosporium* (Fungus)	*Oscillatoria willei* (Alga)
Lactobacillus strains (Bacteria)	*Cinnamomum camphora* (Plant)	*Verticillium* sp. (Fungi)	*Gelidiella acerosa* (Alga)
Pseudomonas stutzeri AG259 (Bacterium)	*Emblica Officinalis* (Plant)	*Aspergillus flavus* (Fungus)	
Corynebacterium sp. SH09 (Bacterium)	*Pelargonium graveolens* leaves (Geranium) (Plant)	*Aspergillus fumigatus* (Fungus)	
Enterobacter cloacae (Enterobacteriacae) (Bacterium)	*Pinus eldarica* (Plant)	*Fusarium oxysporium* (Fungus)	
		Fusarium semitectum (Fungus)	

2.2.3(a) Silver Synthesis Using Fungi

Compared to bacteria, fungi tend to secrete large amounts of protein which can promote huge amounts of production of nanoparticles (Mohanpuria, Rana and Yadav, 2008). There are a few species of fungi that are used to synthesize AgNPs, including *Aspergillus fumigates*, *Fusarium oxysporum*, *Hormoconis resinae* and *Phaenerochaete chrysosporium*. To produce AgNPs, the surface of the fungi cell will trap silver ions and the silver ions will be reduced by the nitrate reductase enzyme that presents in the fungi system. Fungi can be considered as the microorganism that can synthesize AgNPs extracellularly due to extensive secretory components. The main benefit of utilizing the uses of fungi in AgNPs production is fungi can secrete large amounts of an enzyme that is free from cellular protein which can enhance the production of nanoparticles. Gudikandula and C. Maringanti (2016) reported that white-rot fungi from *Pycnoporus* sp. have been utilized for synthesizing silver nanoparticles in malt extract broth that contain 10 g/L glucose and 5 g/L of malt extract. The analysis by UV-vis spectrophotometer demonstrated an absorbance peak at 420 nm for biological methods using fungi species as shown in Figure 2.3. This indicates that fungi contain nicotinamide adenine dinucleotide hydrogen (NADH) dependent nitrate reductase enzyme which function to reduce silver ions into AgNPs.

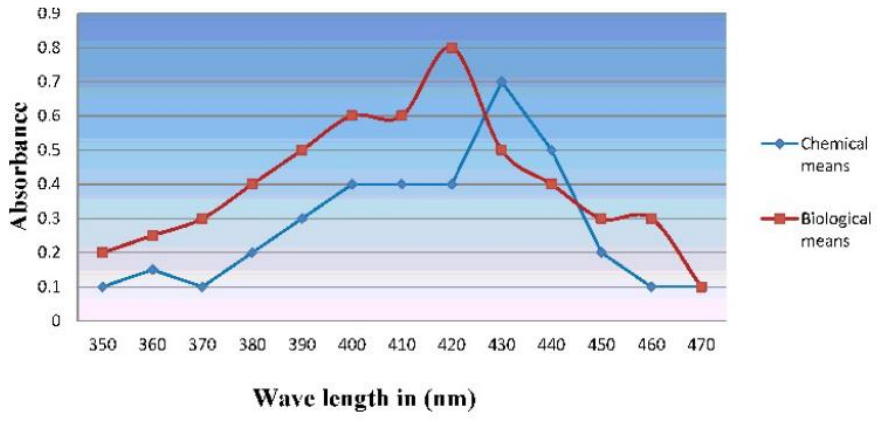

Figure 2.3 UV-Vis Absorption Spectra of AgNPs Synthesised by Both Chemical and Biological Means (Gudikandula and Charya Maringanti, 2016)

2.2.3(b) Silver Synthesis Using Bacteria

Synthetic methods derived from biology have been proved as a potentially viable alternative for metallic nanoparticles production method in recent years. Bacteria are the most common biological organisms that have been used in the synthesis of nanoparticles as this method is cost-effective and greener in approach. Numerous reviews have been published regarding synthesis methods using bacteria and found that there are two types of synthesis methods, namely extracellular synthesis and intracellular synthesis, depending on the location of Ag^+ reduction. Extracellular synthesis can be considered as a cheap method; therefore, more studies are focussed on this method in synthesizing metal nanoparticles. The process of Ag^+ reduction begins when Ag^+ ions get accumulate outside the bacteria's cell wall. The cell that contains the proteins will secrete enzymes that can serve as reducing agents to reduce Ag^+ into AgNPs (Javaid *et al.*, 2017). A study by Lakshmi and Roshmi (2014) reported the extracellular synthesis of AgNPs by using *Bacillus* strain CS 11 isolated from industrialized area, revealed that the studied bacterial strain was found to have resistance towards $AgNO_3$ solution. Silver can be categorized as toxic to bacteria. To counter the toxicity, bacteria will secrete their enzymes to resist Ag^+ ions and remove the toxicity by reducing those particular ions into metal nanoparticles (Javaid *et al.*, 2017). On the other hand, intracellular synthesis of AgNPs involves membrane-mediated silver ion transport into bacterial cells. Some silver-resistant bacteria reduce Ag^+ to AgNPs, which accumulate on the cell wall or in periplasm. However, AgNPs that are formed in the cell wall need to be recovered via some additional steps, including bacteria cell lysis and autoclaving heat treatment. Otari *et al.* (2014) carried an experiment on the intracellular synthesis of AgNPs by actinobacteria *Rhodococcus*

NCIM 2891 and found that there are reductase enzymes that available in actinobacteria cell wall that can facilitate the reduction of Ag^+ ions.

2.3 Biosynthesis Using Plant Extract

Biosynthesis of AgNPs using plants has drawn much attraction since it is an economic beneficial and valuable alternative for large-scale production. This biological approach is not costly and consumed less energy as well as it does not deliver any polluting influences to the environment. For the process of synthesizing nanoparticles, plant extract would be the most preferable reducing agent as it can reduce the consumption of synthesized time and has a high production scale compared to other natural sources (Azmi and Ahyat, 2015). Plant extract can act both as reducing and capping agents and the plant's source may affect the characteristics and morphologies of the synthesized nanoparticles. This is due to different types of plant extracts will produce different concentrations and combinations of reducing agents. Capping agent acts as stabilizers that prevent their coagulation in colloidal synthesis and inhibit the over-growth of NPs (Javed *et al.*, 2020) while reducing agent functions to reduce other component and lose their electron which results in an oxidation reaction. AgNPs are synthesized using plant extracts by bottom-up method at room temperature and atmospheric pressure. In other words, nanoparticles can be generated by independent-assemble of atoms to new nuclei which can form into a nanoscale particle. The biological AgNPs synthesis using plant extract is illustrated in Figure 2.4. Some parts of plants (e.g. leaves, fruits, roots, stem or seeds) can be used for nanoparticles synthesis since phytochemicals is present in the extract. The plant extracts have been found to contain numerous functional groups including alkenyl (C=C), amide (C=N), phenolic and alcohol (O=H), amine (N-H) and carboxylic group (COO-) (Kuppusamy *et al.*,

2016). These chemical substances play important roles in the formation of AgNPs. It appears that the reduction of Ag^+ is caused by metabolite compounds (e.g. polyphenols, alkaloids and amino acids) that are present in the plant. Firdaus *et al.* (2016) has reported there is a large amount of water-soluble organic compounds such as phenolic compounds and ascorbic acid contain in papaya fruit extract that contributed to the ability to reduce Ag^+ to synthesize AgNPs. The plant extract from *Callicarpa maingayi* contains aldehyde groups which are amide and polypeptide which involve in Ag^+ reduction into metallic AgNPs. These functional groups are basically responsible for capping ionic substances into metallic nanoparticles (Shameli *et al.*, 2012).

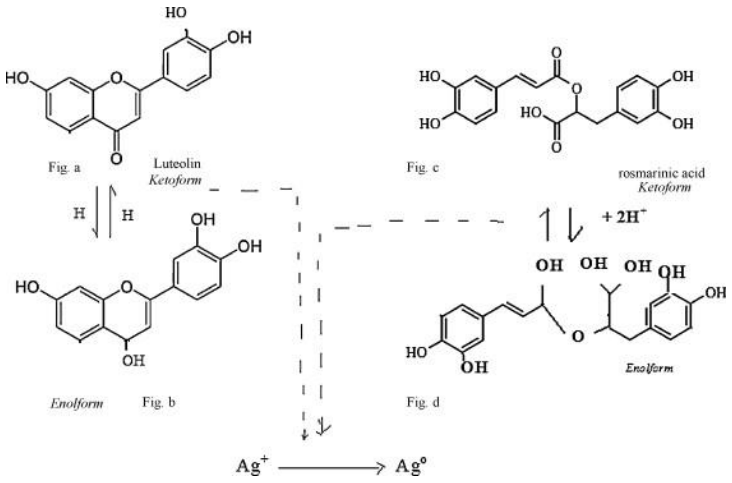

Figure 2.4 Mechanism of Biosynthesis of AgNPs (Shameli *et al.*, 2012)

2.4 *Alpinia galanga*

In the present study, plant extract will be obtained from the rhizome of galangal, *A. galanga* to be used to synthesized AgNPs. *A. galanga* is known as lengkuas in Malaysia is used locally in dishes. This plant belongs to Zingiberaceae family and commonly used to treat the common cold and digestive problems (Raveesha *et al.*, 2021). This plant commonly found throughout the Western Ghats, Mysore, Goa and Gujarat and also found in countries at other regions such as Malaysia, Thailand, Indonesia and China. It has been found to possess various therapeutic activities such as

anti-inflammatory, antifungal and antibacterial (Chudiwal, Jain and Somani, 2010). Moreover, the rhizome part contains tannins and flavonoids such as kaempferol, galangin and alpinin (Chudiwal, Jain and Somani, 2010). Flavonoids are a class of polyphenolic secondary metabolites that plays an important role as a plant defense against herbivore organism. It being reported as a large group of natural substances present in plants that are important to human health due to their high pharmacological activities (Ruiz-Cruz *et al.*, 2017). Humans typically used secondary metabolites as flavoring and medicine. The studies by Siakavella *et al.* (2020) reported that there is a reduction in total phenolic content (TPC) and total flavonoids content (TFC) in the plant extract during the biosynthesis of AgNPs. This result indicates that phenols and flavonoids have been used in the synthesis of AgNPs as a reducing agent. TFC has a lower reduction compared to TPC. Figure 2.5 shows the molecular structure of flavonoids. Tannins, with molecular weights between 500 to 3000 Da, is a group of secondary plant metabolites that are naturally synthesized and accumulated by higher plants. Raja *et al.* (2014) has demonstrated that tannins have been used to biosynthesis AgNPs. In order to produce nanoparticles, 10 mL of concentrated silver and tannins was added together at room temperature (30 °C) and mixed well by using a magnetic stirrer. The result shows that tannins have been involved in Ag^{+} reduction to form AgNPs through hydroxyl groups. The result from FT-IR spectroscopy analysis supported the capping ability of tannins and proved that the functional groups present in tannins are able to reduce Ag^{+}.

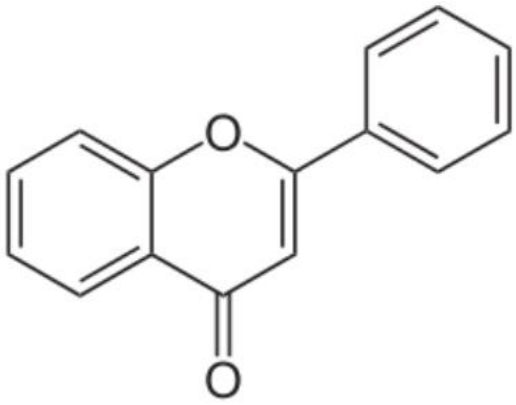

Figure 2.5 *Molecular Structure for Flavonoids*

2.5 Calorimetric Sensing Potential of Silver Nanoparticles

AgNPs had drawn an exciting response from the industry than other types of metals due to their top distinctive localized plasmon surface resonance (LPSR) (Hyder *et al.*, 2022). LPSR is an optical phenomenon that occurs when light interacts with conductive nanoparticles that are smaller than the incident wavelength (Petryayeva and Krull, 2011). The interaction between the incident light and surface electron in a conduction band will generate coherent localized plasmon oscillations with a resonant frequency. This phenomenon is highly dependent on the composition, size geometry, particle-particle separation distances and dielectric environment of nanoparticles which can lead to the change in surface polarization (Petryayeva and Krull, 2011). Unusually strong scattering and absorption properties resulting from this phenomenon make AgNPs greatly efficient at absorbing and scattering light, especially with many dyes and pigments. These properties have made AgNPs as a good environmental sensor towards hazardous heavy metals in solution (Kane, Mishra and Dutta, 2016). Heavy metal refers to any metallic or metalloid element that has a relatively high density (with an atomic density greater than 4 g/cm^3) and it is toxic even at low concentrations.

Colorimetric method is a method for determining the concentration of a chemical element, in this case, is toxic heavy metal, contained in a solution with the aid of nanoparticles. The colorimetric method is an easy and low-cost method since it can be applied without using expensive instruments and heavy metal detection can be visualized by the naked eyes because modifications of their surface charge are transformed into a visible color change (Che Sulaiman *et al.*, 2020; Hyder *et al.*, 2022). AgNPs have the LPSR properties for useful colorimetric assay for the detection of toxic heavy metal ions. Moreover, chemical molecules in the plant extract that has been used to synthesize nanoparticles contain metal interacting multi-functional group such as hydroxyl and carboxyl group that has potential as a colorimetric sensor for hazardous metal ions detection in aqueous solution (Karthiga and Anthony, 2013). The chemical molecules availability in plant extract can be manipulated by controlling the aggregation of AgNPs synthesis.

2.6 Factor Influencing Biosynthesis of Silver Nanoparticles Using Plant Extract

2.6.1 Effect of Reaction Time

The effect of incubation time on green synthesis of AgNPs using plant extract were studied by numerous researchers. Azmi and Ahyat (2015) conducted research on the effect of reaction time (48, 96 and 144 h) and the effect of $AgNO_3$ solution (0.1, 0.01 and 0.001 M) on green synthesis of AgNPs using rhizome extract of *A. galangal*. It was found that the colour intensity of AgNPs solution is highly affected by manipulation of reaction time. At 48 h, the solution's colour changed from colourless to light brown. The colour changed from light brown to brown at 96 h and at 144 h, the colour of solution changed from brown to dark brown. This is due to electron transitions

of surface plasmon resonance in solution which results in formation of AgNPs. The analysis from UV-Vis spectrophotometer found that the stability of AgNPs was increases with the increasing of reaction time from 48, 96 and 144 h. (Nadzir, Idris and Hat, 2019) investigate the effect of reaction time on the formation of AgNPs using *Gynura procumbens* plant extract. The reaction time was varied at 1, 2, 24, 48,72 and 96 h. The results found that the colour changed from yellowish to dark brown with the increasing of reaction time which indicated the formation of AgNPs in the solution. Dissociation of colourless silver ions into AgNPs cause the solution's colour changes to brown. It was reported that the sharp bands of AgNPs occurred at range between 468 to 471 nm.

2.6.2 Effect of Concentration of Silver Nitrate Solution

The concentration of $AgNO_3$ was proven to have effect on AgNPs synthesis process. Through the study by Azmi and Ahyat (2015), different concentration of $AgNO_3$ solution will results in different absorption of SPR bands. The analysis from UV-Vis spectra indicates that the AgNPs synthesized from rhizome extract of *A. galangal* with 0.01 M $AgNO_3$ solution showed the highest absorbance intensity compared to other concentration (0.01 and 0.001 M). The maximum absorbance occurred in range 431 to 438 nm. Nadzir, Idris and Hat (2019) studied effect of $AgNO_3$ solution concentration on the formation of AgNPs using *Gynura procumbens* extract. Different concentrations (4 mM, 8 mM and 12 mM) were tested in this research while maintained concentration of *G. procumbens* extract at 5% (w/v) and ratio of plant extract to $AgNO_3$ solution at 1:50. The result depicted that 12 mM concentration gave the best absorbance at wavelength 452 nm. At higher concentration of $AgNO_3$ used, more AgNPs will be formed due to high amount of Ag^+ available was reduced into

AgNPs. It can be concluded that the absorption peaks of the solution increased with the increasing $AgNO_3$ solution concentration.

CHAPTER 3

METHODOLOGY

This chapter consist of the materials, equipment used and experimental approaches in this research. Each subsection below describes the list of materials and equipment, experimental flowchart and the detailed description of experimental.

3.1 Materials and Equipment

Table 3.1 presents the list of materials used in the experiment and Table 3.2 shows the list of equipment used for the research.

Table 3.1 List of materials

Materials	**Brand**	**Usage**
Alpinia galanga	Online local seller	Sample
Silver nitrate powder	Bendon Laboratory Chemical	Reagent
Metal salt ($MnCl_2$, $NiCl_2$, $CoCl_2$, $FeCl_3$, $FeCl_2$, $AlCl_3$, $Cr(NO_3)_3$, $CdCl_2$, $PbCl_2$, $HgCl_2$, $CuCl_2$)	Bendon Laboratory Chemical	Metal ions solution
Distilled water	-	Solvent

Table 3.2 List of equipment

Equipment	**Brand**	**Usage**
Drying oven	Memmert	To remove moisture of the sample for characterization test
Analytical balance	Shimadzu AC220	To measure mass of the sample accurately
Magnetic stirrer	Fisher Scientific	To mix well the sample
Refrigerator	CoolTech	To store sample for further use
UV-Vis spectrophotometer	Thermo Scientific Genesys-20	To measure the absorbance of samples
Scanning electron microscopy (SEM)	Micromeritics Model ASA 2020	To study the structure of the sample
Zetasizer	Malvern ZEN 3600 Zetasizer	To study zeta potential of the sample
Energy Disperse X-Ray (EDX)	Quanta FEG 450	To analyse the composition of the element in the sample

3.2 Experimental Flow Chart

The proposed research methodology is shown in Figure 3.1.

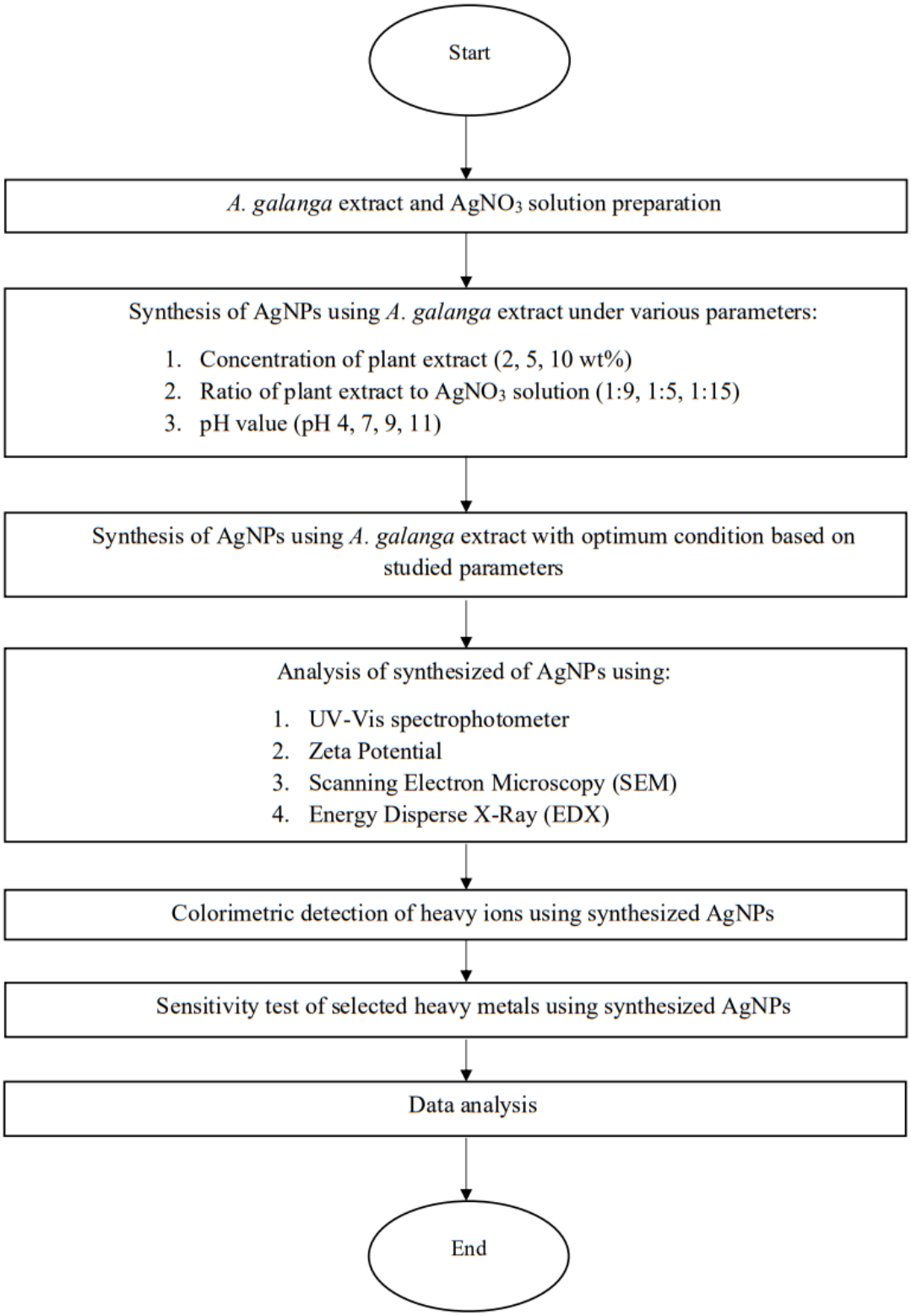

Figure 3.1 Flow diagram of research methodology

3.3 Preparation of *Alpinia galanga*'s Plant Extract

The *A. galanga* extract powder was bought from an online local seller. Aqueous extract of *A. galanga* was prepared at 5 wt% by adding 5 g of *A. galanga* powder into 100 mL of distilled water. The mixture was heated at 80 °C for 10 minutes on a hot plate and stirred at 400 rpm. The solution was then left to cool to room temperature. Then, the mixture was centrifuge at 4000 rpm for 20 minutes before being filtered using Whatman no. 1 filter paper followed by filtration using 0.45 µm syringe filter. The plant extract obtained was stored in a refrigerator for further use.

3.4 Preparation of Silver Nitrate Solution

To prepare 0.01 M concentration of $AgNO_3$ solution, 0.17 g of $AgNO_3$ powder was weighed on an analytical balance before dissolving it in 100 mL of distilled water to produce 0.01 M of aqueous $AgNO_3$ solution. Research by Azmi and Ahyat (2015) on green synthesis of AgNPs using rhizome extract of *A. galangal* reported that AgNPs with 0.01 M $AgNO_3$ concentration demonstrated the highest absorbance intensity compared to 0.001 M and 0.1 M $AgNO_3$ concentration.

3.5 Synthesis of Silver Nanoparticles Using *Alpinia galanga* Plant Extract Under Various Parameters

The parameter studied in this research includes the effect of plant extract concentration, ratio of plant extract to $AgNO_3$ solution and pH value on the formation of AgNPs. The purpose of these parameters is to find the optimal condition for the synthesis of AgNPs before proceed to the characterization of AgNPs. The procedure to conduct the experiment is described in detail as followed. The samples were prepared in triplicates (n=3). The concentration of $AgNO_3$ solution and the reaction time were maintained at 0.01 M concentration and 48 h, respectively. Previous studies found that

the absorbance intensity of AgNPs increased as the reaction time increased. The study conducted by Azmi and Ahyat (2015) on the effect of reaction time (48, 96 and 144 h) on formation on AgNPs synthesized from rhizome extract of *A. galanga* demonstrated that AgNPs with reaction time of 144 h showed the highest intensity of absorbance compared to other reaction time. This was due to the longer reaction time that allowed more reducing agents to interact chemically to reduce the Ag^+ into Ag particles in the mixture (Veerasamy *et al.*, 2011). However, the reaction time was fixed at 48 h for this study due to time constraints. Besides, the result of UV-Vis spectra of AgNPs at different reaction time by Azmi and Ahyat (2015) showed that there was no significant difference between the absorption peaks for 48 h and 96 h reaction time.

3.5.1 Effect of Plant Extract Concentration

Effect of plant extract concentration on AgNPs synthesis using *A. galanga* extract, varied at 5 wt%, 2 wt% and 10 wt% was studied. The concentration of $AgNO_3$ solution and the reaction time were fixed at 0.01 M concentration and 48 h, respectively. The experiment was conducted at room temperature. Firstly, 90 mL of 0.01 M concentration of $AgNO_3$ solution was prepared in a 250 mL beaker and 10 mL of *A. galanga* extract with 5 wt% concentration was slowly added to the mixture and stirred well using a magnetic stirrer. The ratio of plant extract to $AgNO_3$ solution was maintained at 1:9 for this parameter. The mixture was incubated for 48 hours at room temperature. The change in color of the mixture indicated the formation of AgNPs and then was analyzed using UV-Vis spectrophotometer. The experiment was then repeated using 2 wt% and 10 wt% concentrations of *A. galanga* extract. The quality of synthesized AgNPs was investigated and the best concentration of A. *galanga* extract was applied for the subsequent study.

3.5.2 Effect of Ratio of Plant Extract to Silver Nitrate Solution

The effect of the ratio of plant extract to $AgNO_3$ solution on AgNPs synthesis was studied by varying reaction times at 1:9, 1:5 and 1:15. The concentration of $AgNO_3$ solution and the reaction time were maintained at 0.01 M concentration and 48 h respectively. The best concentration for plant extract from the previous study was used in this study. Same steps were repeated as in section 3.5.1 by varying the ratio of plant extract to $AgNO_3$ solution as mentioned above. The mixture's color changes demonstrated the formation of AgNPs was then analysed using UV-Vis spectrophotometer. The quality of synthesized AgNPs was investigated to determine the best ratio of plant extract to $AgNO_3$ solution.

3.5.3 Effect of pH Value

To study the effect of pH value on AgNPs synthesis using *A. galanga* plant extract, the reaction pH value was varied at pH 4, 7, 9 and 11. The reaction pH value was adjusted using 0.1 N HCl and 0.1 N NaOH. Same steps were repeated as in section 3.5.1 and 3.5.2 where the best plant extract concentration and ratio of plant extract to $AgNO_3$ solution from the previous studies were used in this study. The concentration of $AgNO_3$ solution and the reaction time were maintained at 0.01 M concentration and 48 h, respectively. The formation of AgNPs was then analysed through the mixture's color changes and by UV-Vis spectrophotometer.

3.6 Characterization of Silver Nanoparticles

The AgNPs were synthesized once again using *A. galanga* extract at the optimum condition based on previous studies before the characterization process. The formation of AgNPs was confirmed by UV-vis spectrophotometry in a wavelength range of 300-800 nm at a different time interval and also was observed by the color