EXTENDED ESSAY

-Chemistry-

"Green Synthesis of Silver Nanoparticles using bladderwrack *(fucus vesiculosus*) extract as a Reducing and Stabilizing Agent"

How is bio-chemical synthesis of silver nanoparticles from 6mM of $AgNO_3$ solution influenced by change in *(A) concentration of bladderwrack extract* and *(B) temperature*?

Word Count: 3797 (excluding data tables, equations, formulas calculations, figures and their captions: 597 words)

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1 Introduction and Purpose

As a former student athlete and having lived in the Philippines, I often buy sports apparel. In addition to keeping me cool in the tropical weather of the Philippines, what I liked about wearing sports apparel is that they are often designed to protect athletes like me from odors. Over the years, I have seen a common trend on the anti-odor material in the clothes I buy. The use of silver nanoparticles in the clothing industry to protect athletes from odor-causing bacteria has been on the rise.[1] My interest in material science and chemistry has led me to explore the synthesis methods of silver nanoparticles. My exploration introduced me to conventional methods (e.g. UV irradiation, laser ablation, ultrasonic fields, etc.) of synthesizing silver nanoparticles of which turned out to be expensive and requires hazardous chemicals.[2] Thus, it is imperative to find alternative ways of synthesizing silver nanoparticles that are less expensive, non-toxic, and environmentally friendly. One of the trends in the green methods of synthesizing silver nanoparticles is the use of plant extracts. My boarding school is situated next to a fjord in Norway and I noticed that bladderwrack, a species of brown seaweed, is of great abundance. Although the use of many species of brown algae in the synthesis of silver nanoparticles have been investigated[3], bladderwrack's applicability is still undocumented. All of these led me to choose the field of silver nanoparticle synthesis as my topic for my Extended Essay in Chemistry.

1.1 Research Question

General

How is bio-chemical synthesis of silver nanoparticles from 6mM of AgNO3 solution influenced by change in *(A) concentration of bladderwrack extract* and *(B) temperature*?

Specific

A. Is there a significant relationship between the change in absorbance of 6mM of AgNO3 solution with various concentrations of bladderwrack extract (1%, 2.5%, 4%, 5.5%, 7%, 8.5%) and change in temperature?
B. Is there a significant relationship between the change in Absorbance of 6mM of AgNO3 solution and concentration of bladderwrack extract at four temperature (e.g. Room Temperature, 35 degree celsius, 50 degree celsius, 65 degree celsius)?

[1] Fauss, Emma. "The silver nanotechnology commercial inventory." *University of Virginia* (2008).
[2] Kharissova, Oxana V., et al. "The greener synthesis of nanoparticles." *Trends in biotechnology* 31.4 (2013): 240-248.10.1016/j.tibtech.2013.01.003.
[3] Vasquez, Ross D., et al. "Polysaccharide-mediated green synthesis of silver nanoparticles from Sargassum siliquosum JG Agardh: Assessment of toxicity and hepatoprotective activity." *OpenNano* 1 (2016): 16-24.

1.2 Background Chemistry

1.2.1 Silver Nanoparticles and Nanomaterials

Silver nanoparticles have sizes between 1nm - 100nm in size.[4] Silver nanoparticles have unique optical, electrical, and thermal properties.[5] Thus, the application of silver nanoparticles in many fields (e.g. medicine, clothing, wastewater treatment, etc.) has gained popularity over the years. This is mainly related to the antibacterial properties of zerovalent silver and easy reduction of silver(I) salts to form zerovalent silver.[6] Silver nanoparticles can be synthesized in various shapes (e.g. spherical, cubes,etc.) and sizes.[4]

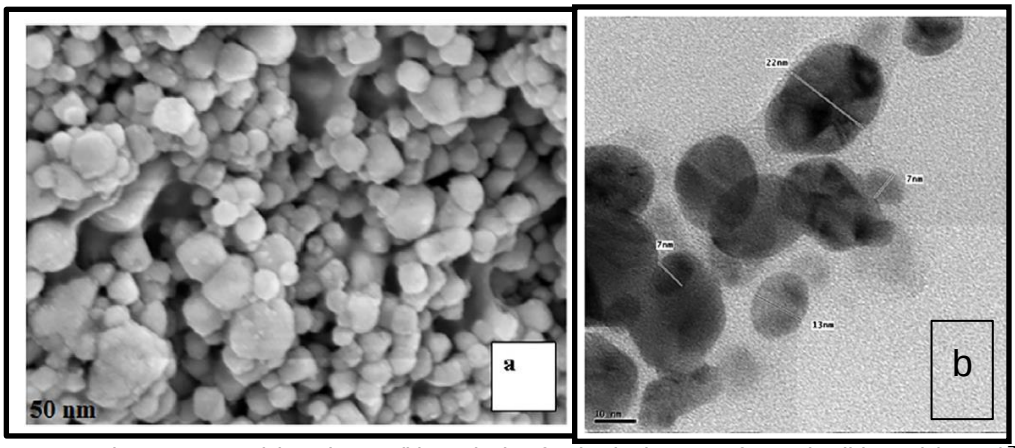

Figure 1. SEM (a) and TEM (b) analysis of spherical AgNPs from AlSalhi, et al (2019.)[7]

1.2.2 Green methods of synthesizing Silver Nanoparticles[8]

As mentioned in the introduction, conventional methods of synthesizing silver nanoparticles are expensive and require hazardous chemicals. In light of this, products from nature or those derived from natural products, such as extracts of various plants

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[4] Graf, Christina, et al. "A general method to coat colloidal particles with silica." *Langmuir* 19.17 (2003): 6693-6700.

[5] *"Silver Nanoparticles: Properties and Applications." Sigma, www.sigmaaldrich.com/technical-documents/articles/materials-science/nanomaterials/silver-nanoparticles.html.* Accessed 27 August 2019

[6] Kharissova, Oxana V., et al. "The greener synthesis of nanoparticles." *Trends in biotechnology* 31.4 (2013): 240-248.10.1016/j.tibtech.2013.01.003.

[7] AlSalhi, Mohamad S., et al. "Synthesis of silver nanoparticles using plant derived 4-N-methyl benzoic acid and evaluation of antimicrobial, antioxidant and antitumor activity." *Saudi journal of biological sciences* 26.5 (2019): 970-978.

[8] Srikar, Sista Kameswara, et al. "Green synthesis of silver nanoparticles: a review." *Green and Sustainable Chemistry*6.01 (2016): 34.

or parts of plants have been used as reductants and as capping agents during synthesis.[9] The main mechanism of action is Redox Reaction which will be discussed later in this chapter.

1.2.3 Bladderwrack and Polyphenols

Bladderwrack and seaweeds in general are known to be rich in polyphenols. This is well documented in a study by Wang, et al. (2019)[10] on *Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds.*

Polyphenols are secondary metabolites of plants and are generally involved in defense against ultraviolet radiation or pathogens. Many research[11] have explored that these molecules are very good antioxidants and may neutralize the destructive reactivity of undesired reactive oxygen/nitrogen species during metabolic processes in the body.

More than 8,000 polyphenols have been identified in various plant species. Polyphenols may be classified into different groups according to the number of phenol rings that they contain and on the basis of structural elements that bind these rings to one another. The main classes include phenolic acids, flavonoids, stilbenes, and lignans.[11]

1.2.4 Reduction and Stabilization Mechanism

Silver nanoparticles are usually grown from Ag+ solutions. From AgNO3 solution, Ag+ ions are grown by the dissociation of Ag from NO3. The grown

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[9] Kharissova, Oxana V., et al. "The greener synthesis of nanoparticles." *Trends in biotechnology* 31.4 (2013): 240-248.10.1016/j.tibtech.2013.01.003.

[10] Wang, Tao, Rosa Jonsdottir, and Guðrún Ólafsdóttir. "Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds." *Food chemistry* 116.1 (2009): 240-248.

[11] Pandey, Kanti Bhooshan, and Syed Ibrahim Rizvi. "Plant polyphenols as dietary antioxidants in human health and disease." *Oxidative medicine and cellular longevity* 2.5 (2009): 270-278.

ions are first reduced to atoms by means of a reducing agent. The obtained atoms then nucleate in small clusters that grow into particles. Depending on the availability of atoms, which in turn depends on the silver salt to reducing agent concentration ratio, the size and shape of the nanoparticles can be controlled. In the synthesis of silver nanoparticles, three elements are needed for the nanoparticle to grow: a silver salt, a reducing agent and a stabilizing agent.[12]

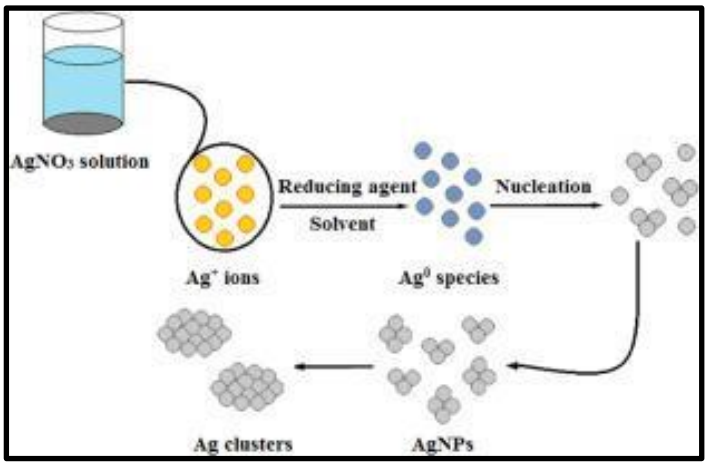


Figure 2. A graphical scheme of the synthesis AgNPs using plant extracts from Datafkhan et al (2013)[13]

Bladderwrack extract has been documented to contain bioactive compounds of which can be used as reducing agents and stabilizing agents. Bladderwrack and brown algae in general have been known to contain phenolic contents[14], gaining the possibility of being used as a reducing agent in biosynthesis of silver nanoparticles from AgNO3. Rodriguez-Leon, et al (2013)[15] explains these molecules are potentially strong reducing agents due

[12] Rodríguez-León, Ericka, et al. "Synthesis of silver nanoparticles using reducing agents obtained from natural sources (Rumex hymenosepalus extracts)." *Nanoscale research letters* 8.1 (2013): 318.
[13] Dastafkan, Kamran, et al. "Mechanism and behavior of silver nanoparticles in aqueous medium as adsorbent." *Talanta* 144 (2015): 1377-1386.
[14] Zenthoefer, Marion, et al. "Isolation of polyphenols with anticancer activity from the Baltic Sea brown seaweed Fucus vesiculosus using bioassay-guided fractionation." *Journal of Applied Phycology* 29.4 (2017): 2021-2037.
[15] Rodríguez-León, Ericka, et al. "Synthesis of silver nanoparticles using reducing agents obtained from natural sources (Rumex hymenosepalus extracts)." *Nanoscale research letters* 8.1 (2013): 318.

to numerous OH groups that promote their antioxidant activity, thus exhibiting free radical scavenging ability as well.

Wright, *et al*., (2001)[16] has proposed two main mechanisms to explain the antioxidant activity of these molecules. In the first mechanism called H-atom transfer, the free radical (R^*) removes a hydrogen atom from the antioxidant (ArOH) that itself becomes a radical:

$$R^* + ArOH \rightarrow RH + ArO^*$$

In the second mechanism known as one-electron transfer proposed by Wright, *et al*., (2001)[16], the antioxidant can give an electron to the free radical becoming itself a radical cation:

$$R^* + ArOH \rightarrow R^- + ArOH^{*+}$$

On the other hand, the most possible mechanism of action has been described by John J, et al (2018)[17] and can be represented by the following chemical equation where the ArOH represents the polyphenolic compound:

$$AgNO_3\,(aq) + ArOH \rightarrow Ag + ArO^- + HNO3$$

From this equation, AgNO3 is reduced to zero valent silver, Ag^0. As illustrated on Figure 2, nucleation of Ag^0 occurs. This, forming silver nanoparticles that are capped by stabilizing agents and therefore regulating the growth of nanoparticles.

[16] Wright, James S., Erin R. Johnson, and Gino A. DiLabio. "Predicting the activity of phenolic antioxidants: theoretical method, analysis of substituent effects, and application to major families of antioxidants." *Journal of the American Chemical Society* 123.6 (2001): 1173-1183.

[17] John, J., C. T. Aravindakumar, and S. Thomas. "Green synthesis of silver nanoparticles using phyto-constituents of Ficus auriculata Lour." *Leaf Extract: Mechanistic Approach. SAJ Biotechnol* 4.103 (2018): 19-21.

In addition, a research study[18] has proven the viability of using Fucoidan, an algal polysaccharide as stabilizing agent in the synthesis of gold nanoparticles by acting as a capping agent.[19] Its mechanism of action, however, is still undocumented.

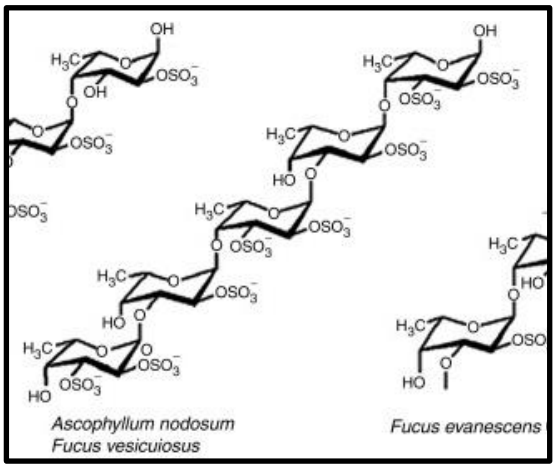


Figure 3. Chemical structure of Fucoidan from fucus vesiculosus from Lim, Seng Joe, and Wan Mustapha Wan Aida (2017).[20]

1.2.5 UV-Visible Spectrophotometer[21]

Spectrophotometry is a method that measures how much a chemical substance absorbs or transmits light. Spectrophotometer measures the amount of photons (the intensity of light) absorbed after it passes through sample solution. In this Extended Essay, I used a UV-visible spectrophotometer that

[18] Ravichandran, Anjali, et al. "Phyto-mediated synthesis of silver nanoparticles using fucoidan isolated from Spatoglossum asperum and assessment of antibacterial activities." *Journal of Photochemistry and Photobiology B: Biology* 185 (2018): 117-125.
[19] Asmathunisha, N., and K. Kathiresan. "A review on biosynthesis of nanoparticles by marine organisms." *Colloids and Surfaces B: Biointerfaces* 103 (2013): 283-287.
[20] Lim, Seng Joe, and Wan Mustapha Wan Aida. "Extraction of Sulfated Polysaccharides (Fucoidan) From Brown Seaweed." *Seaweed Polysaccharides*. Elsevier, 2017. 27-46.
[21] Clark, Jim, and Gamini Gunawardena. "The Beer-Lambert Law." *Chemistry Libretexts, Saatavissa (viitattu 28.5. 2018): https://chem. libretexts. org/Core/Physical_and_Theoretical_Chemistry/Spectroscopy/Electronic_Spectroscopy/Electronic_Spectroscopy_Basics/The_Beer-Lambert_Law* (2007). Accessed 15 July 2019

uses light over the ultraviolet range (185 - 400 nm) and visible range (400 - 700 nm) of electromagnetic radiation spectrum.

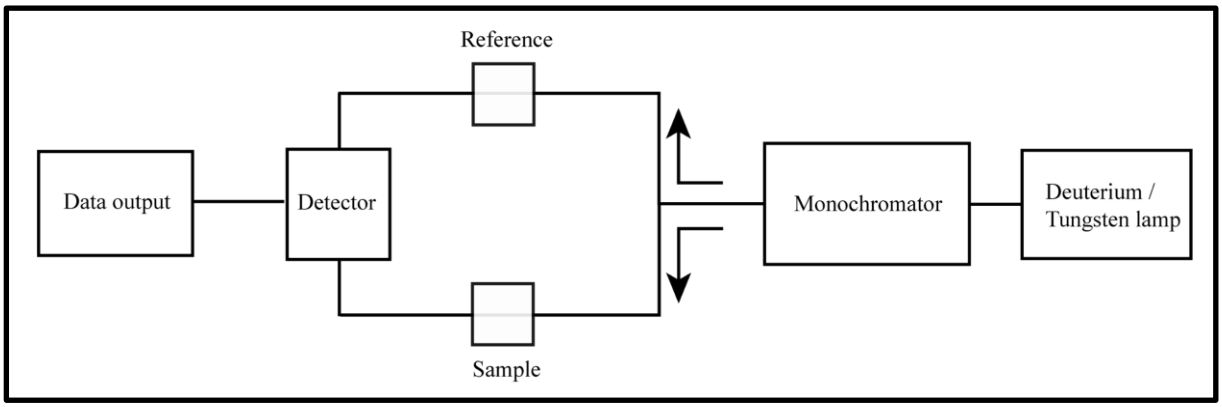


Figure 4. Simplified schematic diagram of a double beam UV–visible spectrophotometer from Wikipedia (2019)[22]

If the intensity of light after it passes through the cuvette is known, it can be related to transmittance (T). Transmittance is the fraction of light that passes through the sample. This can be calculated using the equation:

$$\text{Transmittance(T)} = It/Io$$

Where *It* is the light intensity after the beam of light passes through the cuvette and *Io* is the light intensity before the beam of light passes through the cuvette. Transmittance is related to absorption by the expression:

$$\text{Absorbance(A)} = -\log(\text{T}) = -\log(It/Io)$$

1.2.6 Beer Lambert Law and its limitations

In order to determine the concentration of a substance in a solution, one can use the Beer Lambert Law. Beer Lambert Law shows direct correlation between absorption and concentration of an absorbing species. It is often written as:

[22] "Ultraviolet–Visible Spectroscopy." *Wikipedia*, Wikimedia Foundation, 23 Nov. 2019, en.wikipedia.org/wiki/Ultraviolet–visible_spectroscopy. Accessed 19 July 2019

$$A = \varepsilon * b * c$$

Where,

A - Absorption

ε - Extinction Coefficient

B - Path length

C - Concentration

Beer Lambert Law would help me quantify the concentrations of silver nanoparticles in my sample. However, a difficulty I encountered was determining the Extinction Coefficient of the silver nanoparticles solution as this vary depending on the size and shape of silver nanoparticles. This would only be possible with electron microscopy, of which I did not have access to at my school. Beer Lambert Law also requires me to determine the maximum absorption of my sample, of which was not possible with the UV-Visible Spectrophotometer I used. With respect to the Beer Lambert Law, the assumption I had was an increase in absorption at the usual maximum absorption of silver nanoparticles solution (at 425nm) means there was an increase in concentration of silver nanoparticles in my solution.

1.3 Hypothesis

General

On the assumption that bladderwrack extract contains polyphenols and fucoidan, concentration of bladderwrack extract and increase in temperature will increase the biochemical synthesis of silver nanoparticles from 6mM of $AgNO_3$ solution.

Specific

A. There is a positive significant relationship between the change in absorbance of 6mM of $AgNO_3$ solution with various concentrations of bladderwrack extract (1%, 2.5%, 4%, 5.5%, 7%, and 8.5%) and change in temperature
B. There is a positive significant relationship between the change in Absorbance of 6mM of $AgNO_3$ solution and concentration of bladderwrack extract at four temperature (e.g. Room Temperature, 35°C, 50°C, and 65°C)

1.4 Variables

Table 1: Independent Variable for Research Question A

Independent Variable	
Variable	How will it be changed?
Concentration of bladderwrack extract	Each experimental setup and control setup will have six cuvettes and each cuvette will have a different concentration (**e.g. 1%, 2.5%, 4%, 5.5%, 7%, and 8.5%**) of bladderwrack extract prepared by dilution from 10% aqueous bladderwrack extract.

Table 2: Independent Variable for Research Question B

Independent Variable	
Variable	How will it be changed?
Temperature	There will be four experimental setups and four control setups. Each will be exposed to a temperature (**e.g.**

	20°C/room temperature, 35°C, 50°C, and at 65°C) using Clifton Water Bath.

Table 3: Dependent Variable

Dependent Variable	
Variable	How will the change be measured?
Absorbance of samples	Using a V-1100D Spectrophotometer. See 2.7 V-1100D Spectrophotometer procedure.

Table 4: Control Variables

Control Variable	
Variable	How will it be controlled?
AgNO3 solution concentration	6mM will be prepared and will be used in all experimental and control setups.
Duration and Intervals of data collection	Data collection for absorbance of silver nanoparticle solution will be measured from 15 minutes to 75 minutes (with 15-minute intervals) after preparation and exposure to a temperature. Macroscopic appearance will be observed at the beginning of data collection and after 90 minutes
Wavelength used to determine	425nm was the wavelength used to

absorbance	determine the absorbance of all setups. Silver Nanoparticle solution has been recorded to have a maximum absorbance around 425 by Rodríguez-León, Ericka, et al. (2013)[23]

2 Investigation

The methods done in this extended essay are based on a research study of Vasquez, et al (2015)[24] with some modifications.

2.1 Apparatus

Equipment and Glassware

1. Kern EW, Digital Weighing Scale (±0.01g)
2. V-1100D Spectrophotometer (Wavelength Accuracy: ±2nm, Photometric Accuracy: ±0.5%)
3. Clifton Waterbath
4. Digital Camera
5. (1) 1000ml Volumetric Flask (±80ml)
6. Volumetric Flask Stopper
7. (1) 1000ml Graduated Cylinder (±5ml)
8. (6) Erlenmeyer
9. (1) Beaker
10. Funnel
11. Filter Paper
12. Parafilm
13. 3.5ml Cuvettes, 10mm path length and are 45mm high
14. Chemical Storage Bottle
15. 25ml and 10ml Volumetric Glass Pipette (±0.8ml, ±0.4ml)
16. 10ml Graduated Glass Pipette (±0.1ml)
17. Respirator
18. Gloves
19. Lab Gown
20. Digital Timer

[23] Rodríguez-León, Ericka, et al. "Synthesis of silver nanoparticles using reducing agents obtained from natural sources (Rumex hymenosepalus extracts)." *Nanoscale research letters* 8.1 (2013): 318.
[24] Vasquez, Ross D., et al. "Polysaccharide-mediated green synthesis of silver nanoparticles from Sargassum siliquosum JG Agardh: Assessment of toxicity and hepatoprotective activity." *OpenNano* 1 (2016): 16-24.

Chemicals and Liquids

1. AgNO3 salts from Frederiksen Scientific
2. Deionized Water

2.2 Risk and Safety

As AgNO3 is known to be corrosive and skin irritant, lab gown and gloves were worn at all times when working with AgNO3. On the disposal of AgNO3 and synthesized silver nanoparticles, standard chemical disposal procedure was followed in accordance with international guidelines and the Norwegian Law.

2.3 Plant Collection

Bladderwrack was collected from the fjords in Flekke, Norway. Plant was identified based on published literature on its physical characteristics.

2.4 Extraction Procedure

The collected plant samples were initially washed under running water to remove extraneous materials. Plant samples were washed with deionized water afterwards. An aqueous solution was made by placing 40g of bladderwrack and 400ml of deionized water in a 500ml beaker at 60 degrees celsius for 60 minutes using a water bath. I used 60 degrees and not a temperature higher than that as degradation of useful constituents that is suspected to be responsible for the reduction of silver ions of silver nanoparticles has been documented at a temperature higher than 60°C.[25] Thus, yielding 10% aqueous solution of bladderwrack extract. The aqueous solution was filtered using a filter paper to remove contaminants from the

[25] Rajbhar, K. A. R. I. S. H. M. A., Himanshu Dawda, and Usha Mukundan. "Polyphenols: Methods of extraction." *Sci. Revs. Chem. Commun* 5.1 (2015): 1-6.

solution. A total of 380ml of aqueous solution was obtained. 20 ml of deionized water was lost as a result of evaporation.

Polysaccharides and polyphenols have shown reasonable solubility in water, however, greater solubility have been documented in many other solvents.[25] I used deionized water as my solvent as it is very accessible, and many researches involving plant extracts as bio reductant in the synthesis of AgNPs used the same solvent as well.

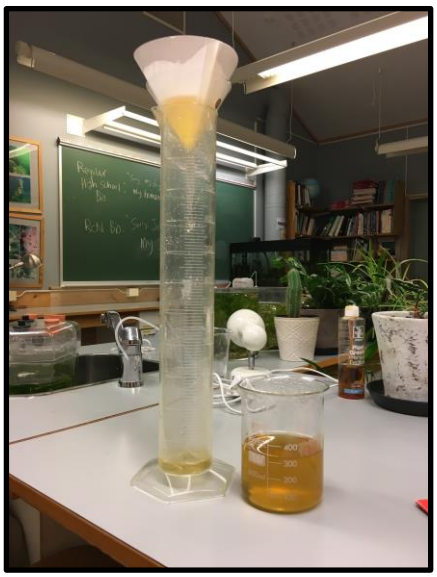


Figure 5. Filtration of aqueous extract solution of bladderwrack

2.5 Bladderwrack dilution of solution preparation

Using the formula $(\%w/v^1)(V^1)=(\%w/v^2)(V^2)$, 6 concentrations (**e.g. 1%, 2.5%, 4%, 5.5%, 7%, and 8.5%**) from 10% of bladderwrack extract were prepared by dilution.

Figure 6. Various dilutions of bladderwrack extract

$$(\%w/v^1)(V^1)=(\%w/v^2)(V^2)$$

Where,

$\%w/v^1$ - weight volume percentage concentration of bladderwrack extracts

V^1 - Volume of bladderwrack extracts

$\%w/v^2$ - weight volume percentage concentration of diluted bladderwrack extracts

V^2 - Volume of deionised water to be added to V^1 for dilution

$$\frac{(\%w/v1)(V1)}{V2} = (\%w/v2)$$

$$\frac{(.1\%w/v)(34\ mL)}{4\ mL} = 8.5\%w/v$$

$$\frac{(.1\%w/v)(28\ mL)}{4\ mL} = 7\%w/v$$

$$\frac{(.1\%w/v)(22\ mL)}{4\ mL} = 5.5\%w/v$$

$$\frac{(.1\%w/v)(16\ mL)}{4\ mL} = 4\%w/v$$

$$\frac{(.1\%w/v)(10\ mL)}{4\ mL} = 2.5\%w/v$$

$$\frac{(.1\%w/v)(4\ mL)}{4\ mL} = 1\%w/v$$

2.6 AgNO3 solution Preparation

1) 16.987g of AgNO3 was weighed using a digital weighing scale
2) The weighted AgNO3 was placed in a 1000ml flask and 500ml of water was poured afterward.
3) The flask was swirled to dissolve all of the Silver Nitrate.
4) 500ml of water was added until the water Meniscus reaches the mark on the flask.

2.7 V-1100D Spectrophotometer procedure

1. V-1100 D was allowed to warm up for at least **20** minutes. The wavelength was set to 425nm **and** was put in Transmittance mode.
2. A blanking cuvette was inserted into the first cuvette holder, the sample compartment cover was closed, set transmittance was set to 0.000% by pressing “0%T” until the display read “0.000”.
3. The holder was pulled to make the blanking cuvette not block the light path, transmittance was set to 100% by pressing “100%T” until the display reads 100.0.
4. Sample solution to be measured was inserted into the cuvette holder. The sample compartment cover was closed, then the sample was pulled into the light path. The results were read directly on the digital display and was tabulated.

Figure 7. Measurement of the absorbance of the samples using V-1100 Spectrophotometer at 425nm

2.8 Uncertainty Calculations

Table 5: Percentage uncertainty of measurements made

Specimen/Solution	Equipment	Absolute Uncertainty	Measured/ Used Value	Percentage Uncertainty
AgNO3 salt (s)	Kern EW, Digital Weighing Scale	± 0.01g	16.987	0.06
Wavelength Used	V-1100D Spectrophotometer	±2nm	425	0.47
AgNO3 solution (aq)	1000ml Volumetric Flask	±80ml	1000	8.00
Plant material	Kern EW, Digital Weighing Scale	±0.01g	40	0.03
Deionised water for plant solution	1000ml Graduated Cylinder	±5ml	400	0.13
Extracted plant extract	1000ml Graduated Cylinder	±5ml	380	1.32

3 Experimentation and Results

3.1 Experimental Group - Synthesis of AgNO3

Biosynthesis procedure was based on a research study of Rodríguez-León, et al (2013)[26] with some modifications. The procedures for the synthesis were optimized to determine the relationship between the concentration of seaweed extract and biosynthesis of silver nanoparticles. The set-up was the reduction of 6 mM of AgNO3 with varying concentration of extract (**1%, 2.5%, 4%, 5.5%, 7%, and 8.5%**) on 1:1 ratio. Four replicates of this setup were made, and each was maintained at a temperature (e.g. 20°C or room temperature, 35°C, 50°C, and at 65°C) for 75 minutes to determine the relationship between temperature and biosynthesis of silver nanoparticles.

3.2 Control Group

In order to rule out the possibility of the color of bladderwrack extract being responsible for the color change of AgNO solutions, various concentrations of bladderwrack extract were also mixed with distilled water and were maintained at four temperatures as well.

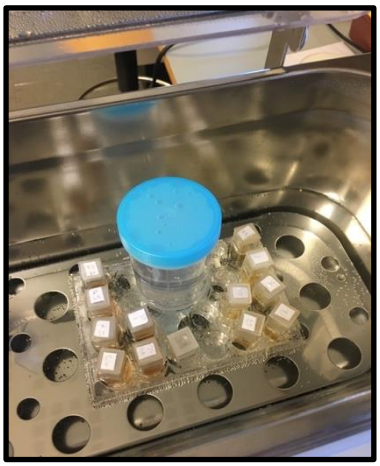


Figure 8. Experimental and Control group in water bath at 35°C

[26] Rodríguez-León, Ericka, et al. "Synthesis of silver nanoparticles using reducing agents obtained from natural sources (Rumex hymenosepalus extracts)." *Nanoscale research letters* 8.1 (2013): 318.

3.3 Data Collection 1: Macroscopic Appearance

The formation of AgNPs was confirmed by the color change of samples. Pictures of the experimental group and control group were taken 90 minutes after preparation of solutions. AgNPs solution usually have a dark brown or bright yellow color.

Table 6: 6mM of AgNO3 with various concentration of bladderwrack extract at 0 minutes and after 90 minutes at **room temperature**

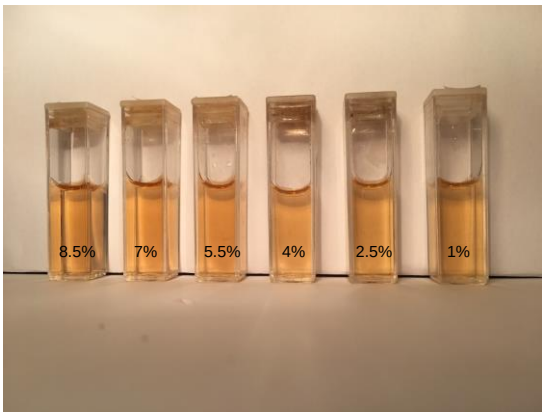
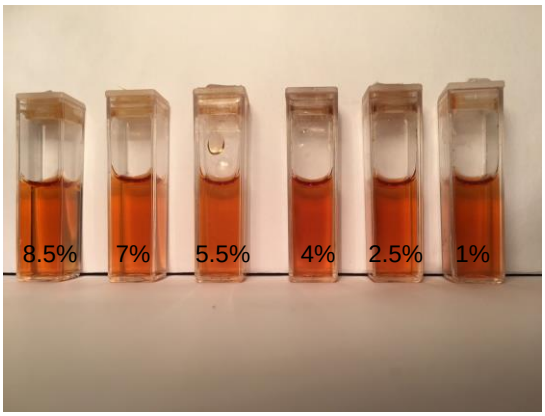
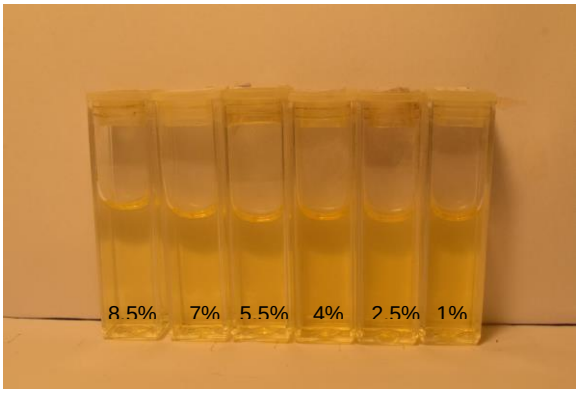
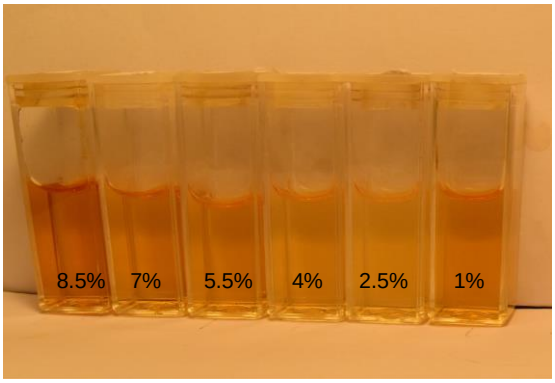
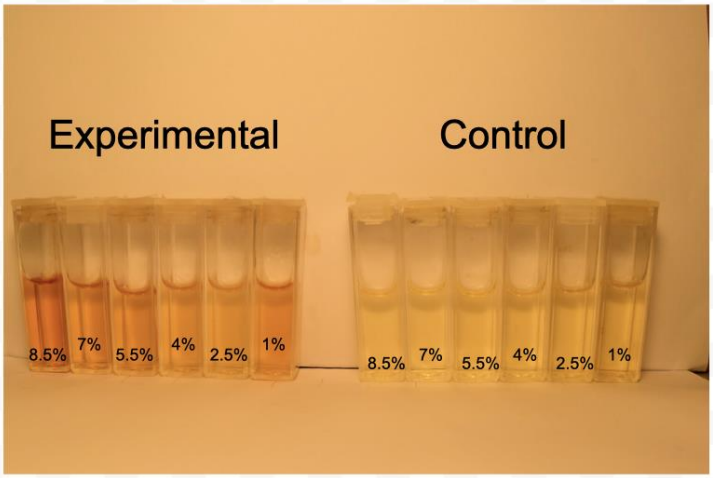
 0 minutes	 90 minutes

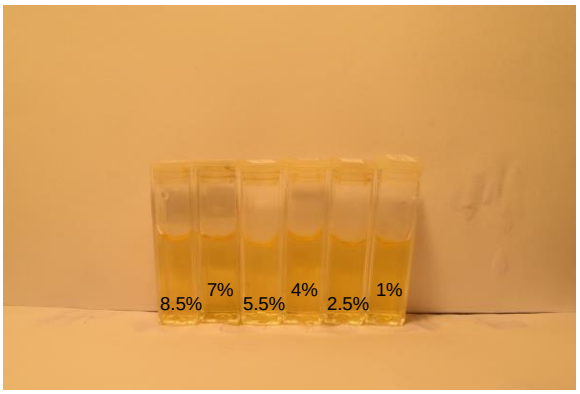
Table 7: 6mM of AgNO3 with various concentration of bladderwrack extract at 0 minutes and after 90 minutes of being submerged to **35°C** water bath

 0 minutes	 90 minutes

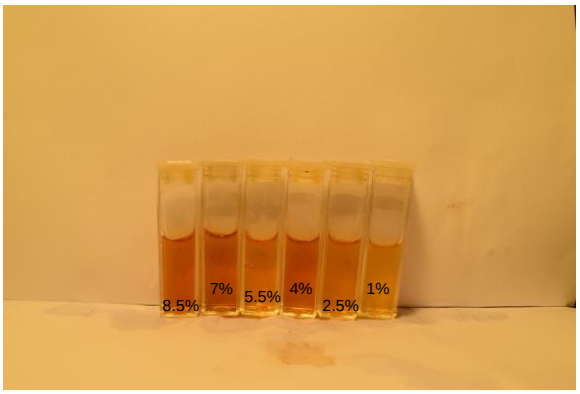


Experimental group and control group after 90 minutes

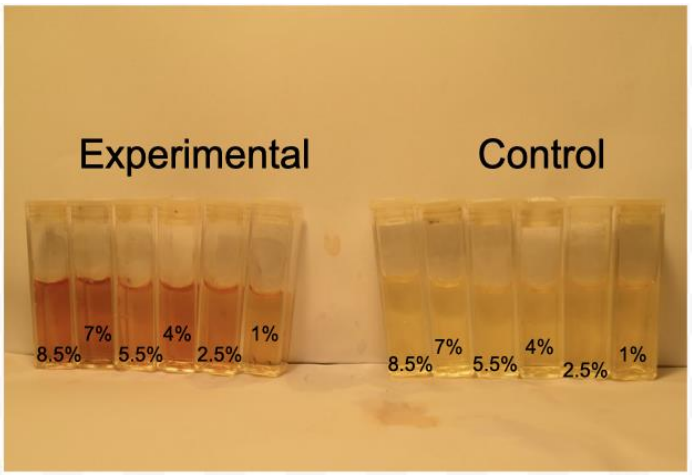
Table 8: 6mM of AgNO3 with various concentration of bladderwrack extract at 0 minutes and after 90 minutes of being submerged to **50°C** water bath



0 minutes

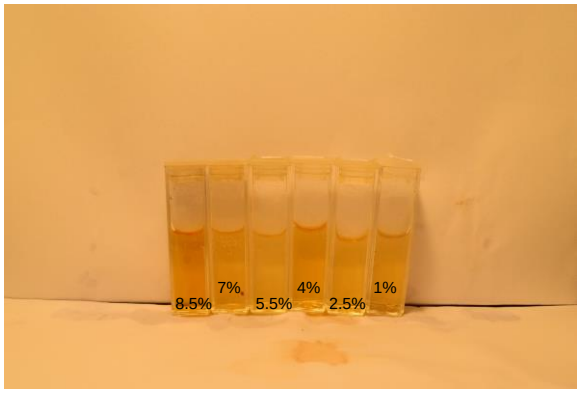


90 minutes

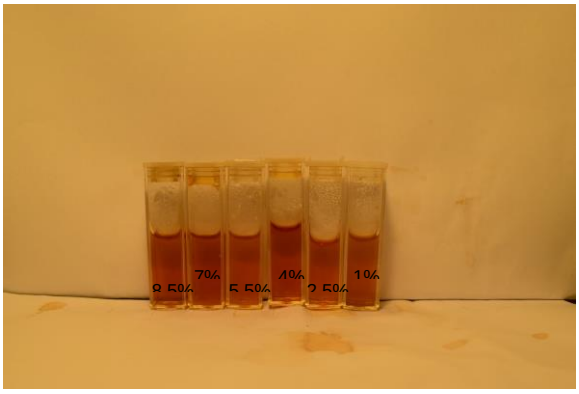


Experimental group and control group after 90 minutes

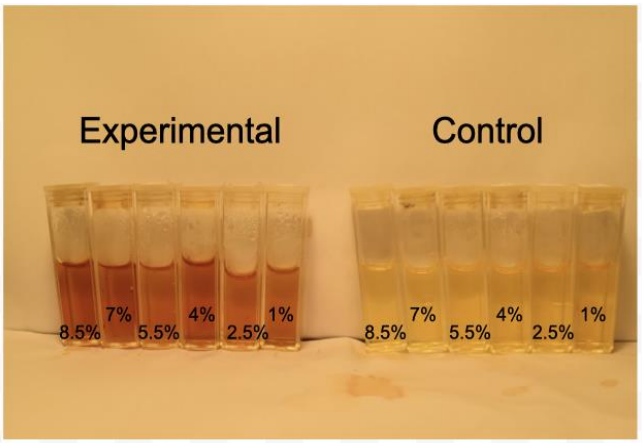
Table 9: 6mM of AgNO3 with various concentration of bladderwrack extract at 0 minutes and after 90 minutes of being submerged to **65°C** water bath



0 minutes



90 minutes



Experimental group and control group after 90 minutes

3.4 Data Collection 2: Absorbance of the Samples

Periodic measurement (for 75 minutes with 15 minutes intervals) of absorbance of AgNO3 with various concentrations of bladderwrack extract at various temperatures using a UV-Vis spectrophotometer at 425nm was done. In addition, periodic measurement of absorbance of distilled water with various concentrations of bladderwrack extract at various temperatures using a UV-Vis spectrophotometer at 425nm was done as well. Collected numerical data for transmittance of experimental group and control group VS. time was tabulated as shown on table 5 and table 6. Absorbance was calculated using the formula described in the introduction.

Table 10: Transmittance and absorbance (at 425nm) of experimental group (6 mM AgNO3 with various concentrations of Algae Extract) at Room Temperature

	Transmittance (±0.5%)							Absorbance						
	Concentration of Algae Extract							Concentration of Algae Extract						Deionized
Time	1%	2.50%	4%	5.50%	7%	8.50%	AgNO3	1%	2.50%	4%	5.50%	7%	8.50%	Water
15 minutes	52.7	47.5	39.8	47.5	49.3	39.6	100	0.28	0.32	0.40	0.32	0.31	0.40	0
30 minutes	30.1	29.9	25.8	23.8	24.3	23.9	100	0.52	0.52	0.59	0.62	0.61	0.62	0
45 minutes	15.7	15.2	13.9	14.2	14.5	12.4	100	0.80	0.82	0.86	0.85	0.84	0.91	0
60 minutes	9.6	9.8	10	9.3	10.3	9.2	100	1.02	1.01	1.00	1.03	0.99	1.04	0
75 minutes	5.5	5.4	5.6	5.1	5.6	5	100	1.26	1.27	1.25	1.29	1.25	1.30	0

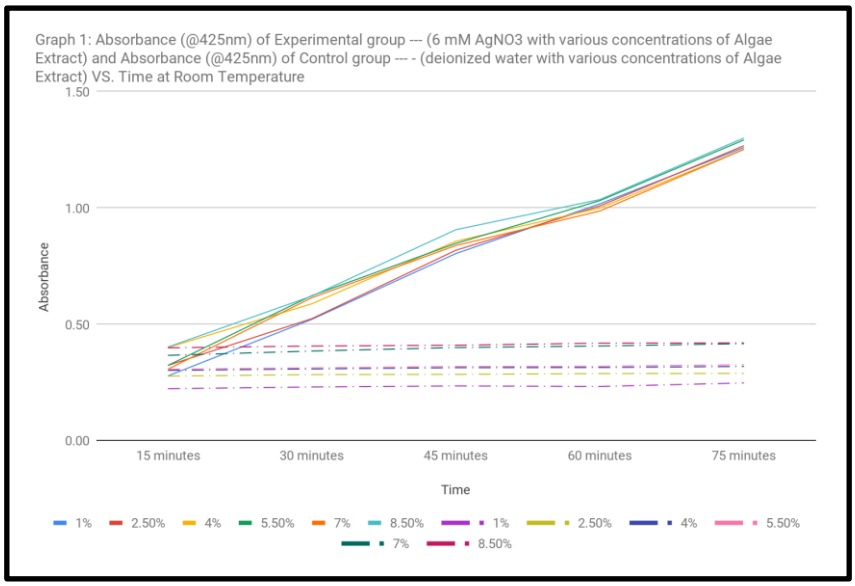
Table 11: Transmittance and Absorbance (at 425nm) of control group (deionized water various concentrations of Algae Extract) at Room Temperature

	Transmittance (±0.5%)							Absorbance						
	Concentration of Algae Extract							Concentration of Algae Extract						Deionized
Time	1%	2.50%	4%	5.50%	7%	8.50%	AgNO3	1%	2.50%	4%	5.50%	7%	8.50%	Water
15 minutes	59.8	52.8	49.9	49.3	43	39.9	100	0.22	0.28	0.30	0.31	0.37	0.40	0
30 minutes	58.8	52	49.2	48.7	41.2	39.2	100	0.23	0.28	0.31	0.31	0.39	0.41	0
45 minutes	58.2	51.9	48.6	48	39.8	38.9	100	0.24	0.28	0.31	0.32	0.40	0.41	0
60 minutes	58.5	51.5	48.5	47.9	39.2	38.1	100	0.23	0.29	0.31	0.32	0.41	0.42	0
75 minutes	56.5	51.4	48	47.2	38.3	38	100	0.25	0.29	0.32	0.33	0.42	0.42	0

The collected data was plotted on a graph as shown in graph 1 on the following page. Table 15, 16, 17, 18, 19, and 20 are attached in the appendix.

3.4.1 Graphical Results 1 for Research Question B

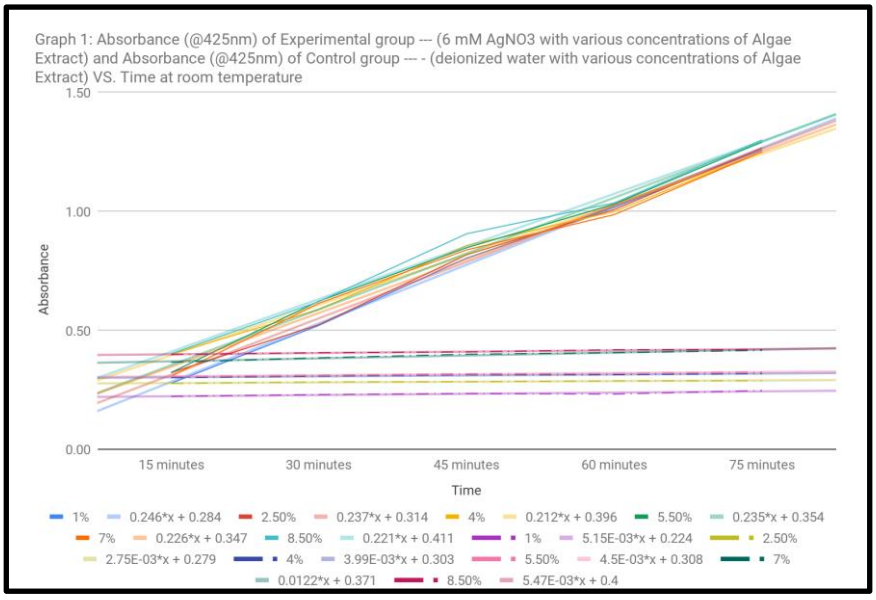
Graph 1 illustrates Absorbance of Experimental group --- and Absorbance of Control group - -- - VS. Time at Room Temperature



Graph 2, 3, and 4 are attached in the appendix.

3.4.2 Data Analysis for Graphical Results 1

A line of best fit drawn on line of absorbance vs. time of each concentration in each experimental and control setup, thus providing a mathematical equation in the form y=mx+b where m is the slope and b is the y-intercept.



Slope of each line was used as the numerical data for the change in absorbance of experimental and control setups. Spearman rank correlation was the statistical tool

used in order to determine the correlation of change in absorbance of experimental & control samples and concentration of bladderwrack extract at particular temperature. Data Analysis was only done on experimental data as control data obviously showed no significant correlation between change in absorbance of control samples and concentration of bladderwrack extract in all graphs.

Table 12: Calculating Spearman Correlation Coefficient to determine the strength of correlation between change in absorbance and change in concentration at room temperature.

Concentration	Change in Absorbance = m	Concentration (Rank)	m (Rank)	d (difference)	d^2
1%	0.246	6	1	5	25
2.50%	0.237	5	2	3	9
4%	0.212	4	6	-2	4
5.50%	0.235	3	3	0	0
7%	0.226	2	4	-2	4
8.50%	0.221	1	5	-4	16
				Sum of all the d^2	58

Using the simplified Spearman Correlation formula, the coefficient was calculated by substituting the the sum of d^2 into the equation:

$$\rho = 1 - \frac{6\sum d_i^2}{n(n^2-1)}$$

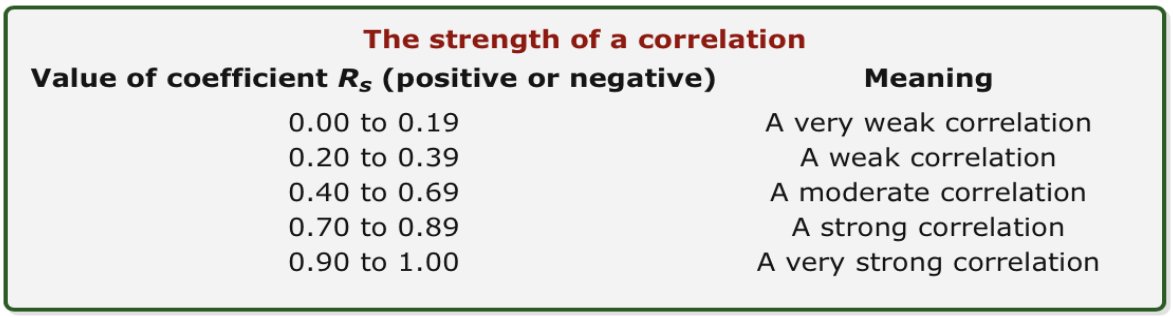
With d^2 equal to 58, and n (number of observations) equal to 6, the formula undergoes the following transformations:

$$p = 1 - \frac{6 * 58}{6 * (6^2 - 1)}$$

P = **-0.65**

Other calculation for the three other temperatures are attached in the appendix.

To interpret the Spearman correlation coefficient, the following guideline was followed:



The strength of a correlation

Value of coefficient R_s (positive or negative)	Meaning
0.00 to 0.19	A very weak correlation
0.20 to 0.39	A weak correlation
0.40 to 0.69	A moderate correlation
0.70 to 0.89	A strong correlation
0.90 to 1.00	A very strong correlation

Figure 9. Spearman correlation coefficient guideline from Barcelona Field Studies Centre (2019)[27]

Table 13: Spearman Correlation Coefficient and Interpretation

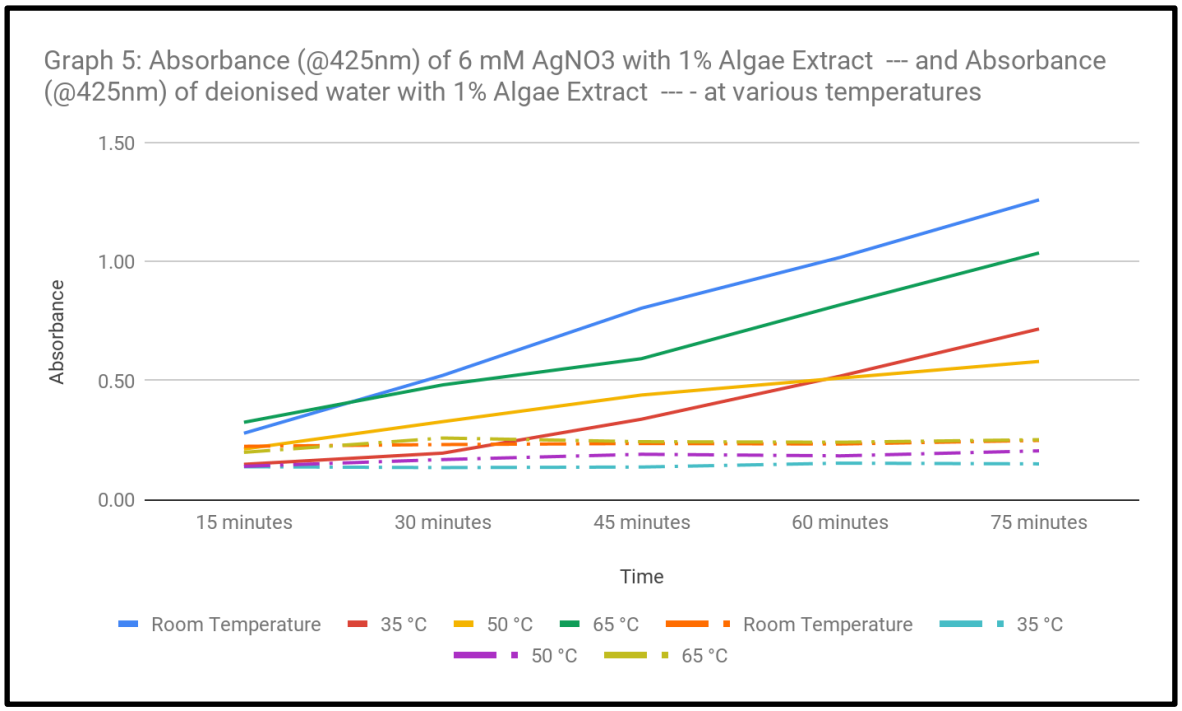
Temperature	Spearman Correlation Coefficient	Strength of correlation between change in absorbance and concentration
At Room Temperature	**-0.65**	moderate negative correlation
35**°C**	-1	a very strong negative correlation
50**°C**	0.94	very strong positive correlation
65**°C**	0.88	a strong positive correlation

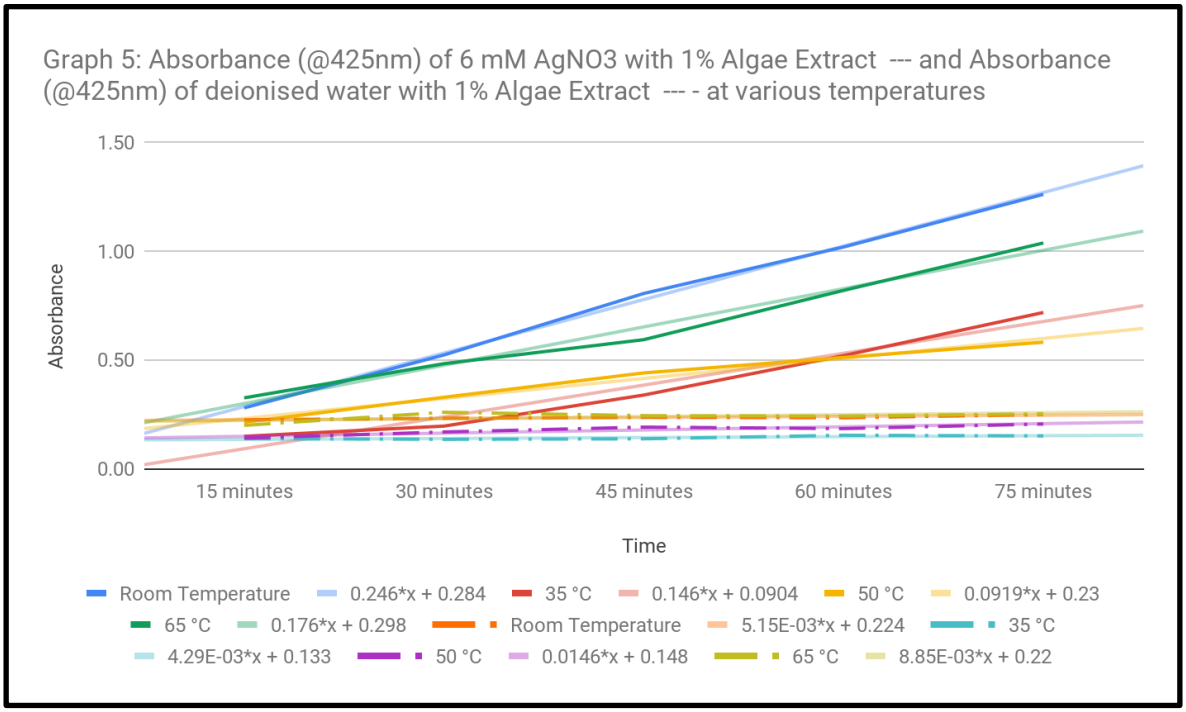
Results of the Spearman correlation showed that there is no positive significant relationship between the change in Absorbance of 6mM of AgNO3 solution and concentration of bladderwrack extract at Room Temperature and 35°C. Whereas, results show there is a positive significant relationship between the change in Absorbance of 6mM of AgNO3 solution and concentration of bladderwrack extract at 50°C and 65°C respectively.

[27] "Spearman's Rank Correlation Coefficient Rs and Probability (p) Value Calculator." *Barcelona Field Studies Centre*, 2019, geographyfieldwork.com/SpearmansRankCalculator.html. Accessed 24 January 2020

3.4.3 Graphical Results 2 for Research Question A

Graph 5 illustrates Absorbance of Experimental group --- (6 mM AgNO3 with a concentration of Algae Extract at various temperatures and Absorbance of Control group --- - (deionized water with a concentration of Algae Extract) VS. Time





Graph 6, 7, 8, 9 and 10 are attached in the appendix.

3.4.4 Data Analysis for Graphical Results 2

Spearman rank correlation was also used as a statistical tool to analyze the correlation between change in absorbance and change temperature at various concentrations.

Table 14: Spearman Correlation Coefficient and Interpretation

Concentration of Bladderwrack Extract	Spearman Correlation Coefficient	Strength of correlation between change in absorbance and temperature
1%	-0.4	moderate negative correlation
2.5%	-0.2	weak negative correlation
4%	-0.2	weak negative correlation
5.5%	-0.2	weak negative correlation
7%	0.8	strong positive correlation
8.5%	0.8	strong positive correlation

Results of the Spearman correlation showed that there is no positive significant relationship between change in temperature and change in absorbance of 6mM of $AgNO_3$ solution with 1%, 2.5%, 4%, and 5.5% of bladderwrack extract. Whereas, results show there is a positive significant relationship between change in temperature and change in absorbance of 6mM of $AgNO_3$ solution with 7% and 8.5% of bladderwrack extract.

4 Conclusion

This Extended Essay demonstrated the ability of extract in synthesizing silver nanoparticles from AgNO3 solutions.

Macroscopic observation confirmed the presence of silver nanoparticles in bulk solution of AgNO3 mixed with different concentrations of bladderwrack extract. All bulk solutions of AgNO3 turned from light yellow color to dark brown indicating formation of silver nanoparticles. Whereas the color of distilled water with brown seaweed remained unchanged, eliminating the possibility of color change in experimental group due to the color of brown seaweed extracts. Positive correlation between time and absorbance of all the experimental samples were also observed as seen numerically and graphically.

Although negative correlation between change in absorbance of experimental samples and temperature was shown by the statistical analysis in low concentration of bladderwrack extract (e.g. 1%, 2.5%, 4%, and 5.5%), a positive trend in the Spearman Correlation Coefficient was observed. The correlation between change in absorbance of experimental samples and temperature becomes more positively significant as concentration of bladderwrack extract increases. Same goes for the correlation of change in absorbance of experimental samples and concentration of bladderwrack extract. Negative correlation between change in absorbance of experimental samples and concentration of bladderwrack extract in low temperatures (e.g. room temperature and 35 degree celsius) was shown by statistical analysis. However, positive correlation between change in absorbance of experimental samples and concentration of bladder wrack extract was shown in 50 and 65 degree celsius.

Therefore, to answer the research question, concentration of bladderwrack extract and temperature do influence the bio-chemical synthesis of silver nanoparticles from 6mM of AgNO3 solution.

The results from this extended essay coincides with the results from many published studies[28,29] on green synthesis of silver particles when it comes to changes in macroscopic characteristics. Thus, demonstrating the potential of this Extended Essay for further investigation.

4.1 Evaluation

As each setup was also done once, the existence of random errors in the absorbance of experimental and control setups are inevitable. This can be avoided by having replicates of every setup. I could have done 3 replicates of my setups, but due the significant amount of time it requires to do each setup I wasn't able to do so. However, the fact that changes in macroscopic characteristics remained consistent in all experimental setups provides sufficient data to answer the research question.

Systematic Errors including the accuracy of the absorbance of experimental and control groups can be eliminated with better equipment. Use of Lab-Scale UV-Visible Spectrophotometer to reduce systematic errors is highly recommended, one that would allow to determine the absorbance of the samples in various wavelengths and therefore confirming the presence of silver nanoparticles in the experimental groups. Use of autoclave could have also eliminated Systematic Error on the temperature the experimental and control groups were exposed to, as heat loss was significant in water bath.

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[28] Vasquez, Ross D., et al. "Polysaccharide-mediated green synthesis of silver nanoparticles from Sargassum siliquosum JG Agardh: Assessment of toxicity and hepatoprotective activity." *OpenNano* 1 (2016): 16-24

[29] Christensen, Laura, et al. "Biosynthesis of silver nanoparticles using murraya koenigii (curry leaf): an investigation on the effect of broth concentration in reduction mechanism and particle size." *Adv Mat Lett* 2.6 (2011): 429-434.

On the other hand, the strength of this study lies on the demonstrated change in macroscopic and absorbance characteristics in the experimental samples. All the experimental samples turned from light yellow color to dark brown and exhibited increased in absorbance at 425nm over 75 minutes. Macroscopic characteristics and absorbance at 425nm of control samples remain unchanged over 75 minutes. Thus, these findings pave way to completely replacing conventional methods that are known to be costly and not environmentally friendly.

4.2 Further Investigation

Isolation and Purification of bioactive compounds in the extracts that is known to act as reducing and stabilizing agents is highly recommended. Although I was able to show empirical evidence of bladderwrack extract's ability to synthesize silver nanoparticles from 6mM of AgNO3, literature on the bioactive compounds in the extract responsible for its reduction and stabilizing capability is still undocumented.

I strongly suggest the investigation on how pH influences the synthesis of AgNPs as many researches[30] have reported its effect on the size and size distribution of AgNPs.

In order to further establish the literature on the use of bladderwrack extract in the green synthesis of AgNPs, use of laboratory-scale characterisation equipment is highly recommended. These include but are not limited to laboratory-scale Uv-Vis Spectrophotometer, Electron Microscopy, and Dynamic Light Scattering.

[30] Singh, Manjeet, I. Sinha, and R. K. Mandal. "Role of pH in the green synthesis of silver nanoparticles." *Materials Letters*63.3-4 (2009): 425-427.

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6 Appendix

Table 15: Transmittance and absorbance (@425nm) of experimental group (6 mM AgNO3 with various concentrations of Algae Extract) at **35°C**

	Transmittance (±0.5%)							**Absorbance**						
	Concentration of Algae Extract							Concentration of Algae Extract						
Time	1%	2.50%	4%	5.50%	7%	8.50%	AgNO3	1%	2.50%	4%	5.50%	7%	8.50%	AgNO3
15 minutes	71.2	60.8	51.8	50.4	44	26.6	100	0.15	0.22	0.29	0.30	0.36	0.58	0
30 minutes	63.9	55	45.8	50.4	40.3	27.3	100	0.19	0.26	0.34	0.30	0.39	0.56	0
45 minutes	46	47.7	42.4	43.9	37.8	24.64	100	0.34	0.32	0.37	0.36	0.42	0.61	0
60 minutes	30.3	40	37.8	38.6	35.6	25.1	100	0.52	0.40	0.42	0.41	0.45	0.60	0
75 minutes	19.2	29.9	27.8	28.4	31.1	22.2	100	0.72	0.52	0.56	0.55	0.51	0.65	0

Table 16: Transmittance and Absorbance (@425nm) of control group (deionized water various concentrations of Algae Extract) at **35°C**

	Transmittance (±0.5%)							**Absorbance**						
	Concentration of Algae Extract						Deionized	Concentration of Algae Extract						Deionized
Time	1%	2.50%	4%	5.50%	7%	8.50%	Water	1%	2.50%	4%	5.50%	7%	8.50%	Water
15 minutes	72.9	70.3	67.5	66.2	66.1	58.9	100	0.14	0.15	0.17	0.18	0.18	0.23	0
30 minutes	73.5	71.9	66.6	65.5	64.5	57	100	0.13	0.14	0.18	0.18	0.19	0.24	0
45 minutes	73.1	72.6	68	65.9	64.2	60.8	100	0.14	0.14	0.17	0.18	0.19	0.22	0
60 minutes	70.4	69.1	65	57.1	60.9	55.6	100	0.15	0.16	0.19	0.24	0.22	0.25	0
75 minutes	70.9	69.1	64.9	55.7	59.5	58	100	0.15	0.16	0.19	0.25	0.23	0.24	0

Table 17: Transmittance and absorbance (@425nm) of experimental group (6 mM AgNO3 with various concentrations of Algae Extract) at **50°C**

	Transmittance (±0.5%)							**Absorbance**						
	Concentration of Algae Extract							Concentration of Algae Extract						
Time	1%	2.50%	4%	5.50%	7%	8.50%	AgNO3	1%	2.50%	4%	5.50%	7%	8.50%	AgNO3
15 minutes	61.4	51	53.1	52.8	49	44.5	100	0.21	0.29	0.27	0.28	0.31	0.35	0
30 minutes	47.1	43.5	43	39.9	38.9	35.1	100	0.33	0.36	0.37	0.40	0.41	0.45	0
45 minutes	36.4	34.1	35.2	26.9	21.9	15.1	100	0.44	0.47	0.45	0.57	0.66	0.82	0
60 minutes	30.9	25.2	25.6	15.4	10.9	7.2	100	0.51	0.60	0.59	0.81	0.96	1.14	0
75 minutes	26.3	16.4	18	9.3	6.2	4	100	0.58	0.79	0.74	1.03	1.21	1.40	0

Table 18: Transmittance and Absorbance (@425nm) of control group (deionized water various concentrations of Algae Extract) at **50°C**

	Transmittance (±0.5%)							**Absorbance**						
	Concentration of Algae Extract							Concentration of Algae Extract						
Time	1%	2.50%	4%	5.50%	7%	8.50 %	Deionized Water	1%	2.50%	4%	5.50%	7%	8.50 %	Deionized Water
15 minutes	72.6	68.3	63.8	62.5	59.3	56.5	100	0.14	0.17	0.20	0.20	0.23	0.25	0
30 minutes	68	64.3	60	59.4	56.5	54.5	100	0.17	0.19	0.22	0.23	0.25	0.26	0
45 minutes	64.6	60.7	57.1	58.3	55.4	49.1	100	0.19	0.22	0.24	0.23	0.26	0.31	0
60 minutes	65.6	61.6	57.6	56.6	54.5	49.1	100	0.18	0.21	0.24	0.25	0.26	0.31	0
75 minutes	62.5	59	55.1	54.8	53.3	46.5	100	0.20	0.23	0.26	0.26	0.27	0.33	0

Table 19: Transmittance and absorbance (@425nm) of experimental group (6 mM AgNO3 with various concentrations of Algae Extract) at **65°C**

	Transmittance (±0.5%)							**Absorbance**						
	Concentration of Algae Extract							Concentration of Algae Extract						
Time	1%	2.50%	4%	5.50%	7%	8.50%	AgNO3	1%	2.50%	4%	5.50%	7%	8.50%	AgNO3
15 minutes	47.4	43.7	44.8	24.7	25.6	27.7	100	0.32	0.36	0.35	0.61	0.59	0.56	0
30 minutes	33	33.5	19.3	18.1	15.8	3	100	0.48	0.47	0.71	0.74	0.80	1.52	0
45 minutes	25.6	21.5	11.1	11.1	5.8	1.8	100	0.59	0.67	0.95	0.95	1.24	1.74	0
60 minutes	15.2	14.6	5.6	7	2.7	0.9	100	0.82	0.84	1.25	1.15	1.57	2.05	0
75 minutes	9.2	9	3.3	3.8	1.4	0.4	100	1.04	1.05	1.48	1.42	1.85	2.40	0

Table 20: Transmittance and Absorbance (@425nm) of control group (deionized water various concentrations of Algae Extract) at **65°C**

	Transmittance (±0.5%)							**Absorbance**						
	Concentration of Algae Extract							Concentration of Algae Extract						
Time	1%	2.50%	4%	5.50%	7%	8.50 %	Deionized Water	1%	2.50%	4%	5.50%	7%	8.50%	Deionized Water
15 minutes	63.4	58.9	56.6	52.3	50.8	47.7	100	0.20	0.23	0.25	0.28	0.29	0.32	0
30 minutes	55.2	56	53	46.7	48.6	45	100	0.26	0.25	0.28	0.33	0.31	0.35	0
45 minutes	57.2	52.9	49.5	44.4	45.1	42.5	100	0.24	0.28	0.31	0.35	0.35	0.37	0
60 minutes	57.5	52.7	47.1	45.2	42.7	41.3	100	0.24	0.28	0.33	0.34	0.37	0.38	0
75 minutes	56.1	51.6	47.5	44.2	43.6	40.5	100	0.25	0.29	0.32	0.35	0.36	0.39	0

Table 21: Calculating Spearman Correlation Coefficient to determine the strength of correlation between change in absorbance and change in concentration at 35°C

Concentration	Change in Absorbance = m	Concentration (Rank)	m (Rank)	d	d^2
1%	0.146	6	1	5	25
2.50%	0.0755	5	2	3	9
4%	0.0624	4	3	1	1
5.50%	0.0614	3	4	-1	1
7%	0.0355	2	5	-3	9
8.50%	0.0194	1	6	-5	25
				Sum of all the d^2	70
				Spearman	-1

Table 22: Calculating Spearman Correlation Coefficient to determine the strength of correlation between change in absorbance and change in concentration at 50°C

Concentration	Change in Absorbance = m	Concentration (Rank)	m (Rank)	d	d^2
1%	0.0919	6	6	0	0
2.50%	0.122	5	4	1	1
4%	0.116	4	5	-1	1
5.50%	0.192	3	3	0	0
7%	0.235	2	2	0	0
8.50%	0.278	1	1	0	0
				Sum of all the d^2	2
				Spearman	0.94285714

Table 23: Calculating Spearman Correlation Coefficient to determine the strength of correlation between change in absorbance and change in concentration at 65°C

Concentration	Change in Absorbance = m	Concentration (Rank)	m (Rank)	d	d^2
1%	0.176	6	5	1	1
2.50%	0.173	5	6	-1	1
4%	0.28	4	3	1	1
5.50%	0.204	3	4	-1	1
7%	0.329	2	2	0	0
8.50%	0.42	1	1	0	0
				Sum of all the d^2	4
				Spearman	0.88571429

Table 24: Calculating Spearman Correlation Coefficient to determine the strength of correlation between change in absorbance of 6 mM AgNO3 with 1% Algae Extract and change in temperature

Temperature	Change in Absorbance = m	Temperature (Rank)	m (Rank)	d	d^2
20	0.246	4	1	3	9
35	0.146	3	3	0	0
50	0.0919	2	4	-2	4
65	0.176	1	2	-1	1
				Sum of all the d^2	14
				Spearman	-0.4

Table 25: Calculating Spearman Correlation Coefficient to determine the strength of correlation between change in absorbance of 6 mM AgNO3 with 2.5% Algae Extract and change in temperature

Temperature	Change in Absorbance = m	Temperature (Rank)	m (Rank)	d	d^2
20	0.237	4	1	3	9
35	0.0755	3	4	-1	1
50	0.122	2	3	-1	1
65	0.137	1	2	-1	1
				Sum of all the d^2	12
				Spearman	-0.2

Table 26: Calculating Spearman Correlation Coefficient to determine the strength of correlation between change in absorbance of 6 mM AgNO3 with 4% Algae Extract and change in temperature

Temperature	Change in Absorbance = m	Temperature (Rank)	m (Rank)	d	d^2
20	0.212	4	1	3	9
35	0.0624	3	4	-1	1
50	0.166	2	3	-1	1
65	0.173	1	2	-1	1
				Sum of all the d^2	12
				Spearman	-0.2

Table 27: Calculating Spearman Correlation Coefficient to determine the strength of correlation between change in absorbance of 6 mM AgNO3 with 5.5% Algae Extract and change in temperature

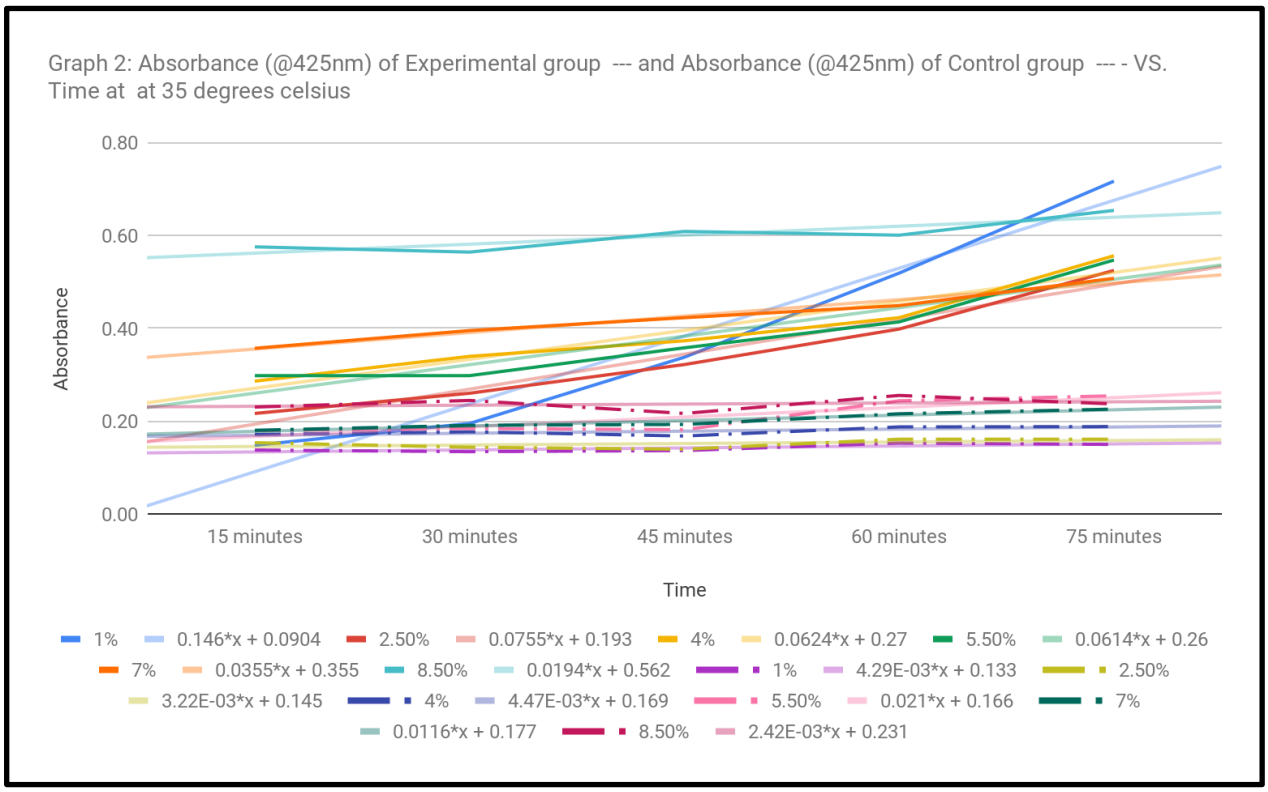
Temperature	Change in Absorbance = m	Temperature (Rank)	m (Rank)	d	d^2
20	0.235	4	1	3	9
35	0.0614	3	4	-1	1
50	0.192	2	3	-1	1
65	0.204	1	2	-1	1
				Sum of all the d^2	12
				Spearman	-0.2

Table 28: Calculating Spearman Correlation Coefficient to determine the strength of correlation between change in absorbance of 6 mM AgNO3 with 7% Algae Extract and change in temperature

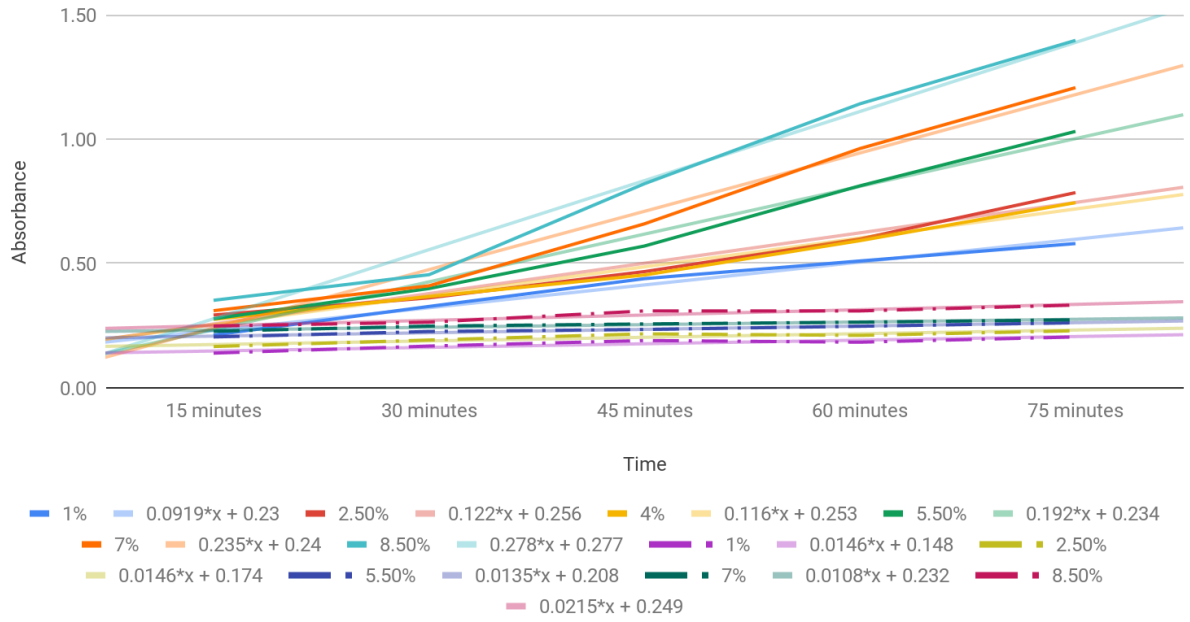
Temperature	Change in Absorbance = m	Temperature (Rank)	m (Rank)	d	d^2
20	0.226	4	3	1	1
35	0.0355	3	4	-1	1
50	0.235	2	2	0	0
65	0.329	1	1	0	0
				Sum of all the d^2	2
				Spearman	0.8

Table 29: Calculating Spearman Correlation Coefficient to determine the strength of correlation between change in absorbance of 6 mM AgNO3 with 8.5% Algae Extract and change in temperature

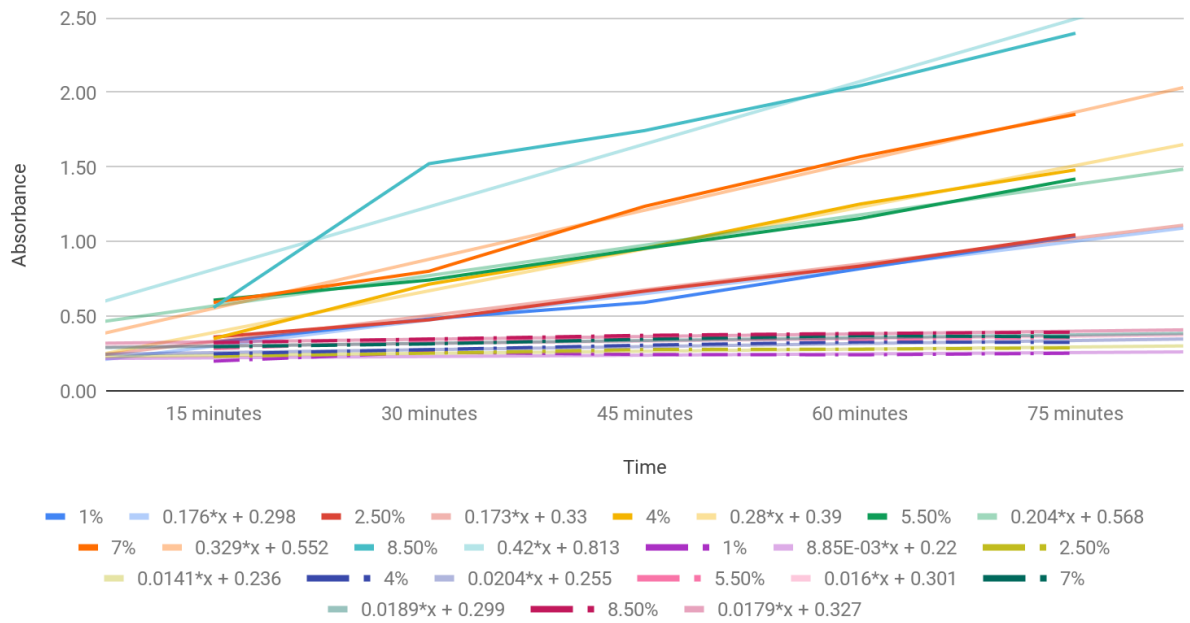
Temperature	Change in Absorbance = m	Temperature (Rank)	m (Rank)	d	d^2
20	0.221	4	3	1	1
35	0.0194	3	4	-1	1
50	0.278	2	2	0	0
65	0.42	1	1	0	0
				Sum of all the d^2	2
				Spearman	0.8



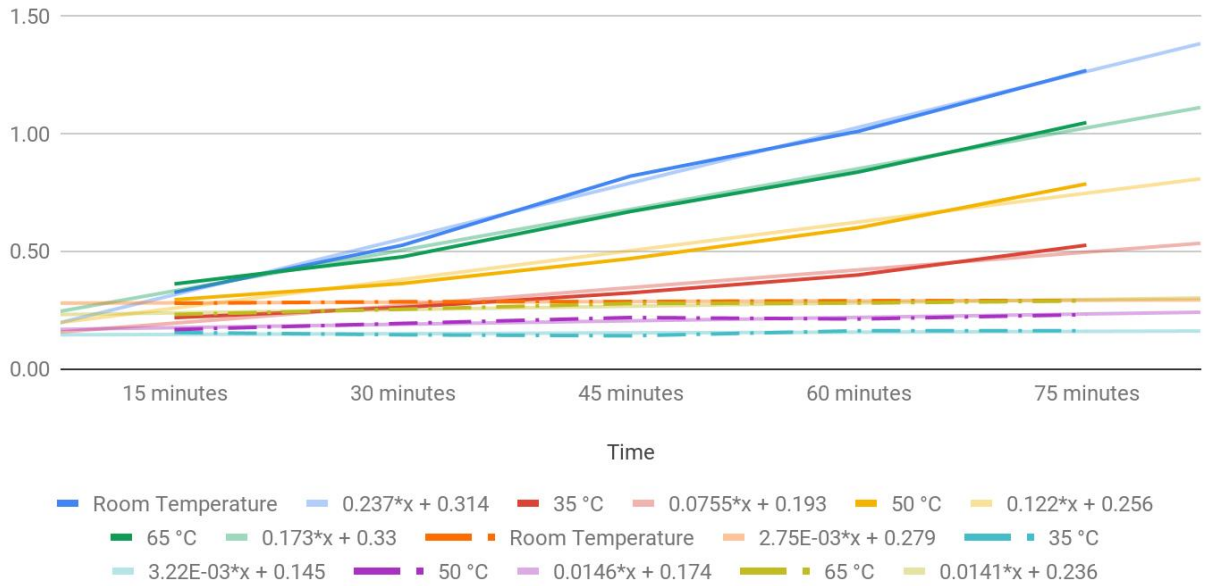
Graph 3: Absorbance (@425nm) of Experimental group --- and Absorbance (@425nm) of Control group --- - VS. Time at 50 degrees celsius



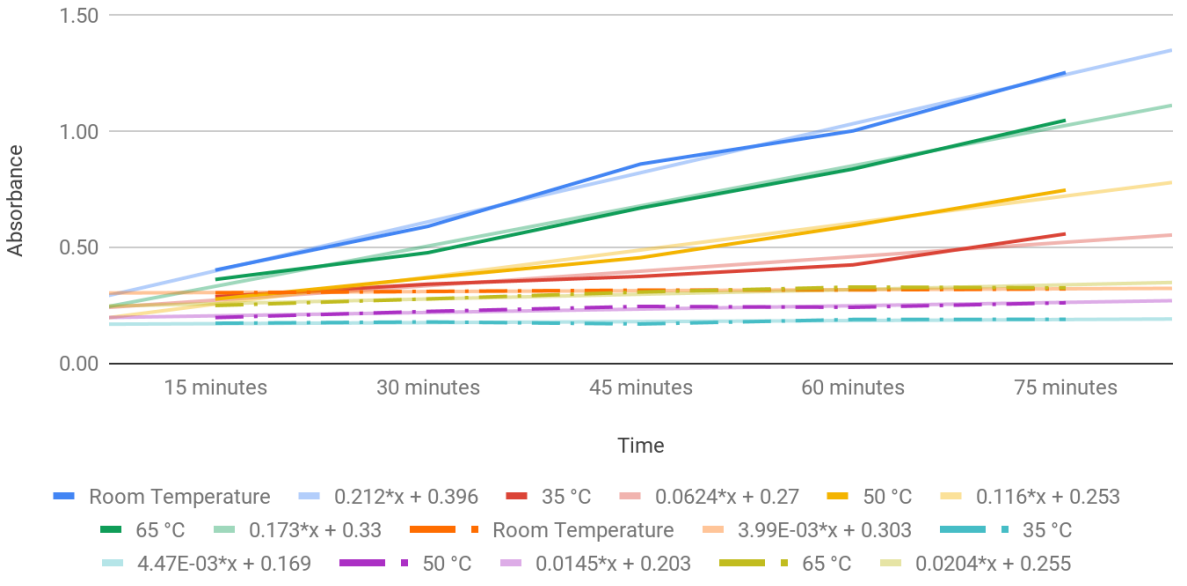
Graph 4: Absorbance (@425nm) of Experimental group --- and Absorbance (@425nm) of Control group --- - VS. Time at 65 degrees celsius



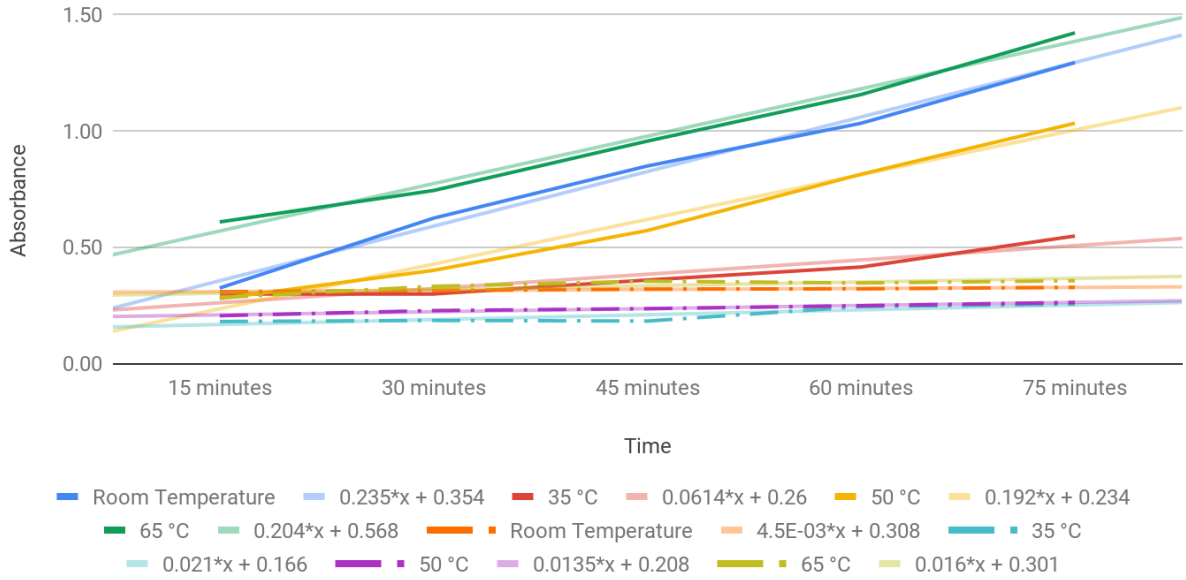
Graph 6: Absorbance (@425nm) of 6 mM AgNO3 with 2.5% Algae Extract --- and Absorbance (@425nm) of deionised water with 2.5% Algae Extract --- - at various temperatures



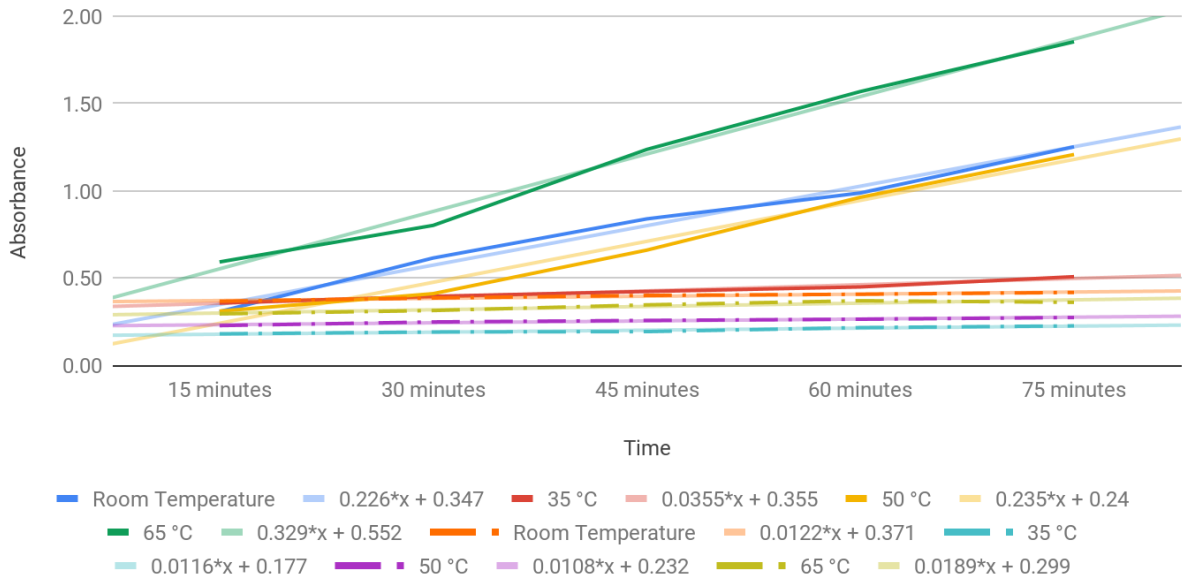
Graph 7: Absorbance (@425nm) of 6 mM AgNO3 with 4% Algae Extract --- and Absorbance (@425nm) of deionised water with 4% Algae Extract --- - at various temperatures



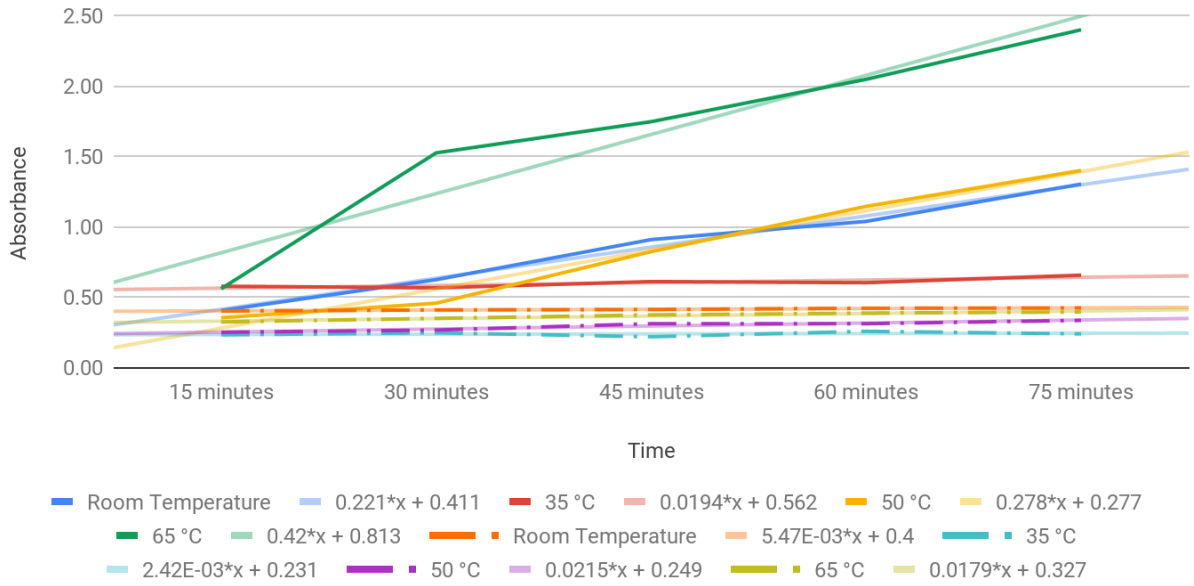
Graph 8: Absorbance (@425nm) of 6 mM AgNO3 with 5.5% Algae Extract ---and Absorbance (@425nm) of deionised water with 5.5% Algae Extract --- - at various temperatures



Graph 9: Absorbance (@425nm) of 6 mM AgNO3 with 7% Algae Extract --- and Absorbance (@425nm) of deionised water with 7% Algae Extract --- - at various temperatures



Graph 10: Absorbance (@425nm) of 6 mM AgNO3 with 8.5% Algae Extract --- and Absorbance (@425nm) of deionised water with 8.5% Algae Extract --- - at various temperatures



Room Temperature 0.221*x + 0.411 35 °C 0.0194*x + 0.562 50 °C 0.278*x + 0.277
65 °C 0.42*x + 0.813 Room Temperature 5.47E-03*x + 0.4 35 °C
2.42E-03*x + 0.231 50 °C 0.0215*x + 0.249 65 °C 0.0179*x + 0.327