

Quantitative Analysis of Liesegang Ring Formation Using Colorimetry: Investigating the Impact of Silver Nitrate Concentrations in a Gelatin-Silver Chloride System

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Abstract: Liesegang Phenomenon is used to describe the formation of periodic precipitation patterns in chemical reactions and was first discovered by the German chemist Raphael Edward Liesegang in 1896. (Liesegang) In a heterogeneous chemical system where a diffusing reagent reacts with another immobile or slowly moving reagent, insoluble precipitates are formed in distinct patterns, also known as Liesegang rings or bands. (Nakouzi) Liesegang rings have been discovered and applied in various scientific and engineering fields, such as materials science, biology, geology, and design. In particular, material engineers are utilizing Liesegang phenomenon to nanomaterials and patterned surfaces to achieve high performance and efficiency with nanoscale precision. (Boudreau) During the development of advanced sensors, catalysts, and photonic materials, the periodic precipitation patterns of Liesegang rings can improve and facilitate the formation of nanostructures with precisely controlled properties, also increasing the yield. Furthermore, in the field of microfabrication, it is extremely useful to apply the Liesegang phenomenon to create periodic structures on surfaces. (Barge et al.) The microfabrication process with periodic structures is fundamental for developing materials with specialized optical or electronic properties and has been employed in many high-tech applications such as semiconductor devices, optoelectronic components, and advanced coating technologies. (Rózsa Szűcs et al.) According to Boudreau, scientists have been successfully designed innovative materials that meet the specific requirements of modern technology by utilizing the inherent periodicity and precision of Liesegang rings, demonstrating the profound impact of this phenomenon on advancing material science and engineering.

Keywords: Liesegang Ring, Gelatin-Silver Chloride System, width law, spacing law, concentration of AgNO_3

1. Introduction

1.1 Research Question

How does the concentration of silver nitrate (AgNO_3) affect the formation, spacing, width of Liesegang rings in a gelatin-sodium chloride system, as analyzed through colorimetric methods?

1.2 Objective

To quantitatively evaluate the effect of different concentrations of silver nitrate on the precipitation of Liesegang rings, using spectroscopy to assess the formation and uniformity of the rings.

A typical Liesegang reaction can be observed using a test tube filled with gel media, in which one of the reagents (the inner electrolyte B) is homogeneously dispersed. An aqueous solution of the other reagent (the outer electrolyte A) is then added on top of the gel column (Nabika et al.). The reaction can be represented by the equation $\text{A(aq)} + \text{B(aq)} \rightarrow \text{P(s)}$, where an insoluble precipitate (P) forms when A meets B. According to Nabika et al., the precipitation process can be explained by mass transport, occurring when the local product of the concentrations of the electrolytes reaches the solubility product, combined with the diffusion of the reagents. Initially, the outer electrolyte diffuses into the gel because its concentration is higher than that of the inner electrolyte. At the interface between the gel and liquid, precipitation occurs immediately due to the high supersaturation of electrolytes, causing the precipitate to grow. (Lagzi and Ueyama) As the local concentration of the inner electrolyte depletes faster than the rate of diffusion, the first band forms. As a result of diffusion flux, where solutes move from regions of

high to low concentration, the inner electrolyte near the first band diffuses further away, lowering its local concentration. The second band forms further away from the first region when the local products of the inner and outer electrolytes again reach the solubility product of the precipitate. This process repeats, resulting in the observation of Liesegang patterns. (Bin Ahmad et al.) We can see the Liesegang Phenomenon in Figure 1.

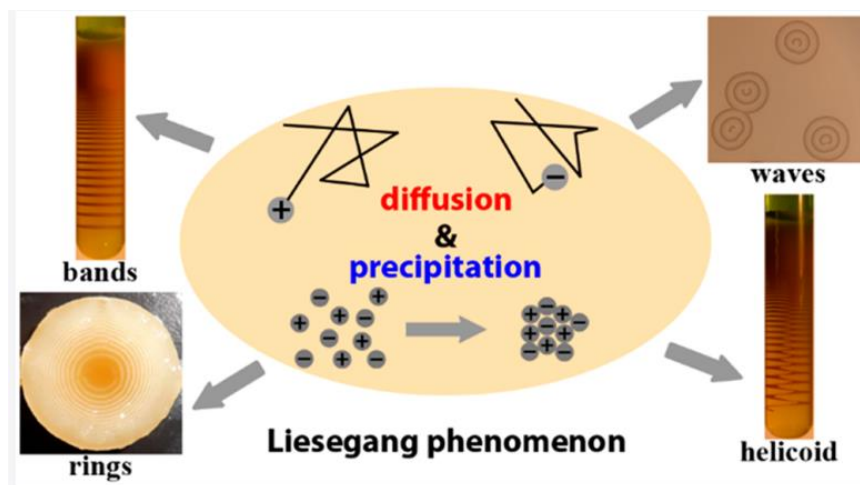


Figure 1- Types of Liesegang Phenomenon (Nabika et al.).

In this experiment, the silver nitrate (AgNO_3) and silver chloride (AgCl) will be used. The silver chloride has a low water solubility with a solubility product K_{sp} of 1.77×10^{-10} , which means only 1.9mg of AgCl is soluble in one liter of water. It is added into the gelatin solution as the inner electrolyte. The silver nitrate is placed above the gelatin layer and played as the role of outer electrolyte. During the reaction, the silver chloride will form as precipitate in the interface between gelatin and silver nitrite. After repeating the process of diffusion flux, a white layer of silver chloride precipitation will occur. Due to the light sensitivity of AgCl and AgNO_3 , they are used in black-and-white photography for producing images. (Stulik)

Gelatin is a multi-functions substance derived from collagen, usually founding in animal bones and connective tissues. The application of gelatin has been developed in many fields, such as food industry, and biomedical application. In this experiment, gelatin is an important reactant to prevent instabilities of formed colloidal particles and precipitates. (Grzybowski et al.) Also, a distinct formation of precipitation band can be created due to the viscosity of gelatin, slowing down the movement of molecules.

2. Hypothesis

Variations in the concentration of silver nitrate (AgNO_3) are hypothesized to affect not only the spacing and number of Liesegang rings but also their color intensity and uniformity, as measurable through colorimetric techniques.

The concentration of AgNO_3 directly affects the diffusion rate of silver ions into the gelatin-sodium chloride system. Higher concentrations create a steeper concentration gradient (Boenigk), accelerating diffusion and promoting denser Liesegang rings due to increased precipitation events. This also alters local supersaturation, with higher concentrations exceeding the precipitation threshold more often, leading to more nucleation sites and narrower ring spacing (Dietrich). Larger silver nitrate concentrations form bigger precipitation particles, resulting in wider rings, while enhanced uniformity in precipitation boosts color intensity and sharpness (Mueller et al., Hadalgeri et al.). These effects make Liesegang rings more distinct and analyzable through spectroscopy.

2.1 Independent Variables

- Concentration of Silver Nitrate (AgNO_3): Different concentrations (e.g., 0.02M, 0.05M, 0.1M, 0.15M, 0.2M) were chosen to observe how varying addition of AgNO_3 influence the formation of Liesegang rings.

2.2 Dependent Variables

- Spacing Between Liesegang Rings: The distance between successive precipitate rings.

$$\lim_{n \rightarrow \infty} \frac{x_{n+1}}{x_n} = 1 + p$$

- where x is the distance of the ring from the interface, n is the number of rings formed, p is the spacing coefficient
- $\Delta x_n = x_{n+1} - x_n$ increases linearly as a function of x_n
- Bands become more separated from each other as x increases
- p can be determined from different concentration of $[\text{AgNO}_3]$

According to the spacing law or Matalon–Packter law (Jablczynski), if x_n marks the distance of formation of the n th band from the initial interface of the dichromate with the silver nitrate, then the ratio $\frac{x_{n+1}}{x_n}$ is a constant. This ratio is normally greater than one (regular patterning) but can occasionally be less than one, resulting in what is known as revert patterning (Antal et al.). The spacing coefficient p can be determined from different concentrations of AgNO_3 .

- Width of Liesegang Rings: The thickness of each individual ring.

$$w_n \propto x_n^\alpha$$

- where w is the width of the ring, let $q = \frac{w_{n+1}}{w_n}$
- α is the exponent: $q = p^\alpha$
- average of q is calculated from spacing law, $\alpha = \log_p q$
- α is usually between 0.9 – 1 (Karpati-Smidroczi et al.)

According to the width law, if w_n denotes the width of the n th precipitate zone, then the ratio $\frac{w_{n+1}}{w_n}$ forms a geometric series of zone thickness. The exponent α is calculated as $q = p \times \alpha$, where q is the average of $\frac{w_{n+1}}{w_n}$, and α is width coefficient usually between 0.9 and 1. (Büki et al.)

- Color Intensity of the Rings: As measured by a colorimeter or spectrophotometer. The use of microscopic picture and spectrophotometer can help distinguished different rings formation.

- Number of Rings Formed: Total count of distinct rings developed in the reaction.

2.3 Control Variables

These factors must be kept constant to ensure that changes in the dependent variables are due to the independent variable alone:

- Volume of the Gelatin-NaCl Mixture: The volume of the gel medium in each test tube must remain consistent. Ensuring the same volume across all samples guarantees that silver ions spread the same distance, maintaining uniformity and preventing differences in diffusion rates that could impact the formation and spacing of Liesegang rings.

- Temperature: The experiment should be conducted at a constant temperature, as temperature fluctuations can affect the diffusion rates of silver ions and the kinetics of the chemical reactions involved in Liesegang ring formation. Maintaining a consistent temperature will lead to more reliable data.

- pH of the Medium: If applicable, the pH should remain consistent across all samples to avoid variations in the reaction environment.

- Size and Shape of the Containers: Test tubes or containers of the same size and shape should be used to ensure uniform diffusion conditions.

- Initial Concentration of Sodium Chloride (NaCl) in Gelatin: This should be uniform across all test tubes.

- Distance Between Camera and Test Tubes When we're Taking Pictures: To ensure the same magnification is used for different photos to accurately compare patterns. These are the factors that need to be kept constant to ensure that any changes in the dependent variables are due to the change in the independent variable only.

3. Methodology

3.1 Materials

- Gelatin 50g
- Sodium Chloride (NaCl) for the gel medium 0.5844g
- 50 mL Silver Nitrate (AgNO_3) solutions of varying concentrations (0.02M, 0.05M, 0.1M, 0.15M, 0.2M)
- DI Water
- Acetone/ethanol for wash up
- Test tubes
- Balance (0.0001g accuracy)
- Volumetric flasks 50mL, 100mL, 250mL, 1L
- Hot plates
- High Resolution Camera
- Tripod
- Standard lab equipment for measurement and safety
- Stir bar
- Parafilm
- Optical Microscope
- UV/Vis spectrophotometer

3.2 Procedure

3.2.1 Gel Preparation of Gelatin-NaCl

In a 1L volumetric flask, 50g of gelatin was mixed with 1L of DI water. The mixture was stirred and heated at 50°C for 25 mins until fully dissolved. 0.5844g NaCl was added into the mixture and heated for another 5mins. 5ml of mixture was poured into test tubes and cooled down to room temperature so the gel was set firmly for at least 3 hours.

3.2.2 Addition of Silver Nitrate

Various concentration of AgNO_3 solution (0.05M, 0.8M, 0.1M, 0.15M, 0.2M) was prepared and diluted to the required concentration. 3ml of 0.2M AgNO_3 solution was added slowly on top of the gel medium and covered with parafilm. This step was repeated for different concentrations in other test tubes. Pictures were taken with high resolution camera after addition.

3.3.3 Quantitative Data Collection

After 3 days, pictures of test tubes were taken at the same position. Spacing and width of the rings were measured and compared.

3.3.4 Analysis of Color intensity and Concentration of AgCl Precipitate

UV/Vis spectrometer was used to measure the color intensity and determination of concentration of AgCl rings formed. Small samples of the precipitated material from the rings were extracted carefully and diluted with known amount of aqueous ammonia. The spectrometer was calibrated with known concentration of AgCl (0.02M, 0.05M, 0.1M and 0.2M). The spectrum was screened between 250nm-

900 nm. Measurement was repeated for samples extracted from various concentration of AgNO_3 -AgCl gelatin systems. Peak intensities were compared to determine the color intensity and concentration of AgCl Liesegang rings formed in the system.

4. Safety and Environmental Considerations:

- Adhere to safety protocols, especially when handling silver nitrate. (PubChem)
- Protective gear should be worn all the time, such as gloves, safety goggles, and lab, since silver nitrate has some corrosive properties. Experimenters should avoid the silver nitrate contact with skin as much as possible.
- The storage of silver nitrate should keep away from the light as it's light-sensitive, and can decompose. In the end, the experimenter should dispose the waste properly due to the hazardous components.
- Instrument Handling: Follow the manufacturer's instructions and safety guidelines for using the spectrophotometer.

5. Result & Discussion

Width of AgCl precipitation is summarized in Table 1 and Figure 2 below:

Table 1. Width of precipitation resulting from different concentration of AgNO_3 added

Test tube number	Concentration of AgNO_3 added (M)	width of precipitation (mm) $\pm 0.5\text{mm}$
1	0.02	23
2	0.05	40
3	0.1	53
4	0.15	60
5	0.2	66

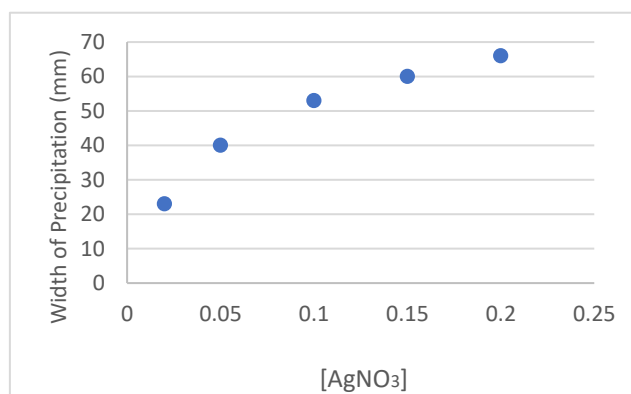


Figure 2. Width of precipitation vs. AgNO_3 concentration

Precipitation was clearly observed in each test tube after 5 days. The width of the precipitation band increased as a function of concentration.

Distinct precipitation bands were visible in each test tube after 5 days, indicating that chemical reactions occurred within the solutions. As the concentration of the substances in the test tubes increased, the width of the precipitation band also increased. The correlation between concentration and the extent of the chemical reactions taking place during the process has been observed. As higher concentrations led to more rigid movements of particles, wider bands of precipitates were formed. This phenomenon can be explained by the increase in collision frequency of reactant particles and effective collisions taken place. The likelihood of successful collisions and subsequent reaction occurrences increased with more reactant particles presented in a given volume. In addition, this experiment has demonstrated a fundamental aspect of solubility behavior - saturation. As substances reach their saturation point at specific concentrations, any excess amount will no longer dissolve but form solid precipitates instead.

This principle has important implications not only in chemistry but also in various industries such as pharmaceuticals and environmental monitoring.

In conclusion, during 5 days of continuous observation, we have witnessed clear evidence of precipitation formation in each test tube with different silver nitrate observation. The width of precipitation varied depending on the concentration used for each sample. This finding highlights how concentration plays a crucial role in determining both reaction rates and solubility behaviors within chemical systems.

In order to investigate the spacing and width of each precipitate ring, analysis was taken by observing the participation area using optical microscope. (Nikon SMZ-745 Stereo Microscope) Images were summarized below for comparison in Figure 3.

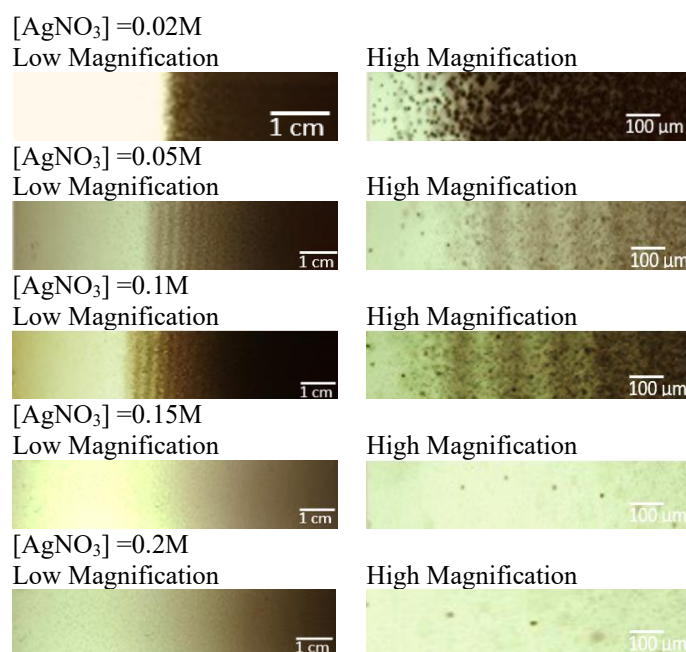


Figure 3. Microscopic Images of Precipitation Area with Various $[AgNO_3]$

At higher concentration above 0.15M, it was also difficult to identify the precipitation front. Only continuous precipitation was observed.

However, at 0.05M and 0.1M, repeated precipitation patterns were observed with low magnification. Reaction interface and band could be identified. Widths and spacings of each band were measured using high magnification microscopic images and summarized in Table 2.

Table 2. Spacing and Width of Precipitation Bands Formed with $[AgNO_3] = 0.05M$ and $0.1M$

$[AgNO_3] = 0.05M$	1 st band	2 nd band	3 rd band	4 th band
Spacing x (μm)	374	489	637	828
Width w (μm)	78	97	86	112
$[AgNO_3] = 0.1M$	1 st band	2 nd band	3 rd band	4 th band
Spacing x (μm)	307	394	512	664
Width w (μm)	87	124	103	133

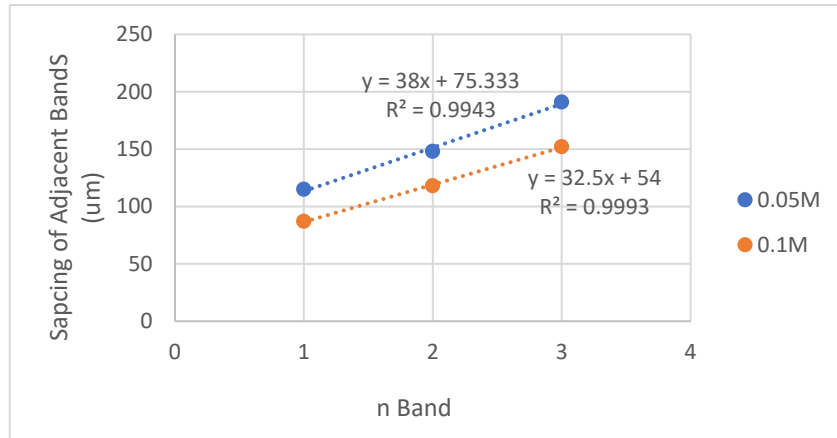


Figure 4. Spacing Effect of Adjacent Bands with $[AgNO_3] = 0.05M$ and $0.1M$

From Figure 4, we have found that $\Delta x = x_{n+1} - x_n$ increased linearly with the increasing number of bands.

Spacing coefficient: $1 + p = \frac{x_{n+1}}{x_n}$ is constant between adjacent bands

$$1 + p = 1.3033 \text{ when } [AgNO_3] = 0.05M$$

$$1 + p = 1.2932 \text{ when } [AgNO_3] = 0.1M$$

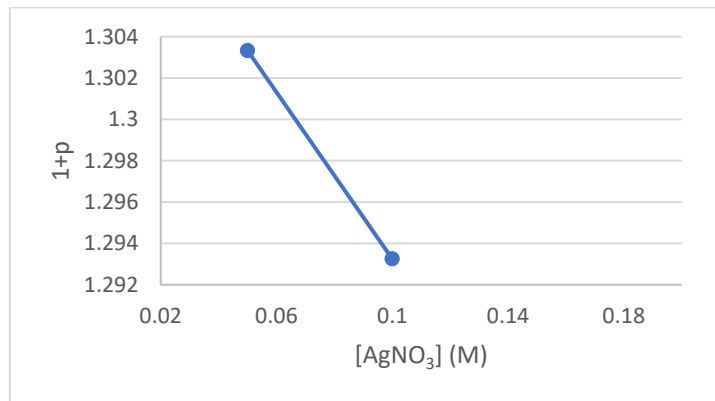


Figure 5. Spacing Coefficient for Different $[AgNO_3]$ Calculated from Experiment

It did agree with literature (Sakamoto et al.) as concentration of $AgNO_3$ increased, the spacing coefficient decreased. Since the spacing coefficient $1 + p > 1$, we can find in Figure 5, the precipitation was proven to be regular-type Liesegang phenomenon.

In order to investigate the width law, $q = \frac{w_{n+1}}{w_n}$ was calculated and plotted in Figure 6 below:

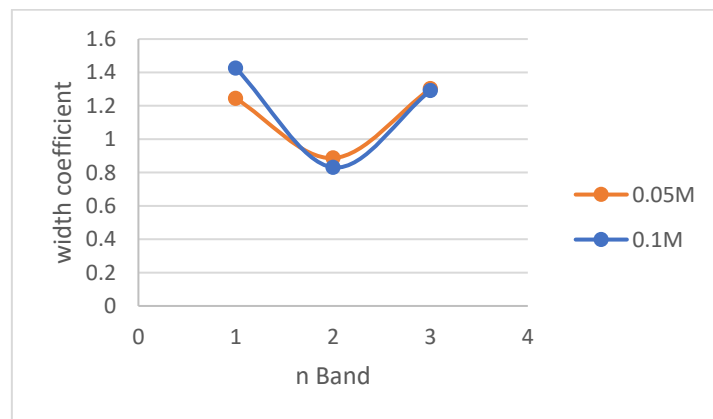


Figure 6. Width Coefficient for Different $[AgNO_3]$ Calculated from Experiment

By theory, the width coefficient should be the constant between adjacent bands (Büki et al.). However, due to the fact that bands were difficult to identify unless higher resolution images were captured, the width law was hard to prove. From literature, the width law is weak to be proven as clear identification of bands are almost impossible to achieve.

6. Conclusion & Evaluation

Although the results of the experiment were successful, there were areas where improvements could have been made to enhance the investigation. The experiments could be evaluated in the following aspects.

First, one of the strengths of this experiment was the validation of the spacing law. Our system followed the regular type of Liesegang formation, where the spacing law states that as the concentration of silver nitrate increases, the spacing coefficient decreases. As shown in Table 3, the spacing coefficient was 1.3033 for 0.05M AgNO_3 and 1.2932 for 0.1M AgNO_3 . The spacing coefficient decreased by 0.01 as the silver nitrate concentration increased by 0.05M, which was consistent with the spacing law. This indicated that the experiment successfully produced Liesegang rings through the silver nitrate-gelatin system.

Second, the observation of Liesegang rings under the optical microscope was highly dependent on the concentration of reactants in the system. It was found that [1] distinctive patterns only emerged at specific concentrations. When the concentration was either too low or too high, no visible pattern formation occurred. In our experiment, distinct ring patterns were observed only in the tubes with silver nitrate concentrations of 0.05M and 0.1M. The tubes with other concentrations did not show clear Liesegang ring patterns under the microscope, making it difficult to collect specific data on spacing and width. This limitation affected the calculation of the spacing law and width law, as only two sets of data could be utilized. Since proving the relationship between concentration and spacing coefficient required more data points, calculations from just two concentrations were insufficient to provide a valid result. To improve the experiment, a wider range of silver nitrate concentrations, especially between 0.05M and 0.1M, should have been tested. For example, adding 0.06M, 0.07M, 0.08M concentration of silver nitrate could have provided more comprehensive data for analysing the spacing and width law.

Third, the time law was not investigated in this experiment. According to the time law, the ratio of $(x_n)^2$ to t_n , where t_n is the time for n th precipitation bands to formed, will approach to a constant, as the increase of n . This mathematical relationship was discovered by Morse and Pierce follows directly from the diffusion-controlled nature of the phenomenon. (Morse)[2] However, the time law is a significant characteristic of Liesegang rings, as it describes the relationship between the time of band formation and the spacing of the bands. To address this issue, a camera could have been set up to record the formation of the bands in the tube at regular intervals, such as every 24 hours. Analyzing the video footage would have allowed us to determine the time of formation and the corresponding ratio.

7. Further Research

Quantitative analysis of AgCl precipitates with different $[\text{AgNO}_3]$

In order to determine the concentration of AgCl formed in the reaction, UV/Vis spectroscopy was used in Figure 7. 0.2M AgCl standard solution was prepared by dissolving 1.4334g AgCl in 50ml of aqueous NH_3 . The solution was then diluted to prepare 0.02M, 0.05M, 0.1M standards. Calibration standards were placed in UV/Vis Spectrometer (UV-1800, Shimadzu) to determine the calibration curve. The chosen wavelength was from 250nm to 900nm.

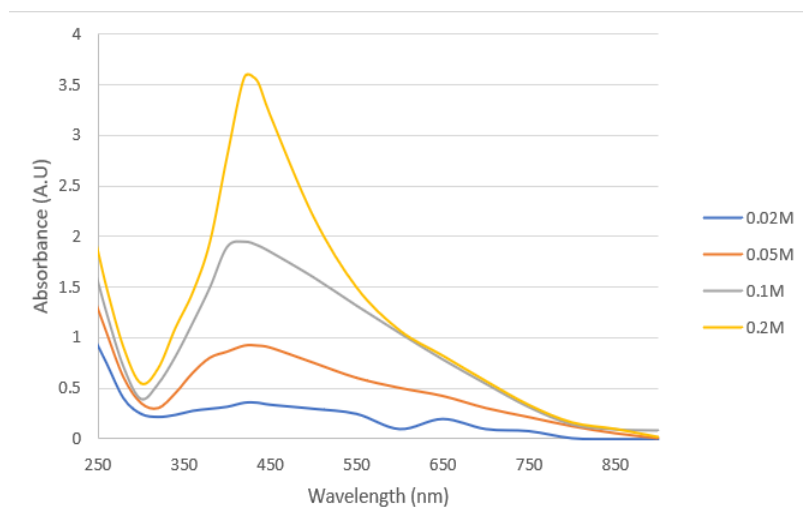


Figure 7. UV/Vis Spectrum of AgCl Standards

From the spectrum, the peak of interest was identified to be around 425nm, which corresponding to Ag/Ag⁺ peak. The calibration curve was constructed in Figure 8 below:

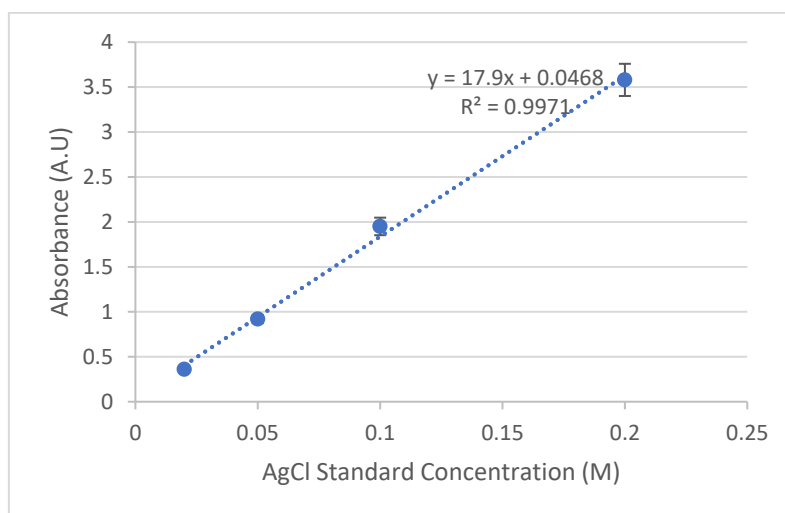


Figure 8. Calibration curve of AgCl standards

Table 3. Absorbance Data at Peak of Interest

Cuvette	Description	Absorbance at 425nm
1	0.02M Standard	0.3697
2	0.05M Standard	0.9226
3	0.1M Standard	1.9574
4	0.2M Standard	3.5718
5	Unknown from 0.02M AgNO ₃	0.8157
6	Unknown from 0.05M AgNO ₃	1.5495
7	Unknown from 0.1M AgNO ₃	1.9472
8	Unknown from 0.15M AgNO ₃	2.1247
9	Unknown from 0.2M AgNO ₃	2.2459

From the spectrum, peaks at 425nm could be distinguished which can be showed in Table 3 . Using the calibration curve, the unknown concentration could be calculated from:

$$\text{Absorbance} = 17.9 \times [\text{Unknown}] + 0.0468$$

$$[\text{Unknown}] = \frac{\text{Absorbance} - 0.0468}{17.9}$$

Table 4. AgCl Concentration Calculated from UV/Vis Spectrum

Test Tube	Description	AgCl Concentration (M)
1	Unknown from 0.02M AgNO ₃	0.043
2	Unknown from 0.05M AgNO ₃	0.084
3	Unknown from 0.1M AgNO ₃	0.106
4	Unknown from 0.15M AgNO ₃	0.116
5	Unknown from 0.2M AgNO ₃	0.123

From UV/Vis in Table 4, when concentration of AgNO₃ added to the tube increased, more Ag⁺ free ions will react with Cl⁻ ion and diffused down the tube to form Liesegang pattern. This finding also consistent with the observation of increased width of precipitation area in each test tubes as discussed previously. The peak at 425nm was the Ag⁺ peak, which is consistent with literature. [3](Anh et al.) However, it would be suggested to use XRD, XEM to investigate the precipitate microscopic structure and the possibilities for Ag⁺ nanoparticles formation.

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