

Extraction and Spectrophotometric Characterization of Papain from Carica Papaya for Application in Nano-Edible Coating

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Abstract

Papain, a proteolytic enzyme obtained from the latex of immature fruits of *Carica papaya*, has wide applications in the food and pharmaceutical industries due to its protein-degrading ability. The study focused on the extraction, spectrophotometric characterization, and formulation of papain-based nano-edible coatings for postharvest applications. Papain was extracted using the latex tapping method, followed by drying to obtain powdered enzyme. A stock solution was prepared and the working concentrations of ($10\text{--}60\text{ }\mu\text{g mL}^{-1}$) were formulated from it. Spectrophotometric analysis was performed over a wavelength range of $200\text{--}800\text{ nm}$ to evaluate absorbance at different concentrations. Maximum absorbance observed in the ultraviolet region was $274\text{--}277\text{ nm}$, and 277 nm was selected as the analytical wavelength due to consistent maximum absorbance across concentrations.. A linear increase in absorbance was observed within the concentration range of $10\text{--}50\text{ }\mu\text{g mL}^{-1}$, confirming adherence to the Beer-Lambert law, while slight deviation at higher concentrations suggested possible protein aggregation effects.

Nano-edible coatings were developed using Response Surface Methodology (RSM) based on Central Composite Design (CCD) to evaluate the effects of oil, surfactant, and co-surfactant on particle size and polydispersity index (PDI). The developed formulation exhibited particle size ranging from $205.4\text{--}466.6\text{ nm}$ and PDI values between $0.30\text{--}0.45$, indicating moderate uniformity of the nano-emulsion system. Statistical analysis of the model was significance. The improved nano-scale characteristics enhanced coating uniformity, adhesion, and barrier properties. Overall, the study demonstrates that optimized papain-based nano-edible coating have strong potential as an effective strategy for improving postharvest stability, quality, and shelf-life of fruits.

1. INTRODUCTION

Papain is a proteolytic enzyme extracted primarily from the latex of immature fruits of *Carica papaya* [1, 22]. It belongs to the group of cysteine proteases and has wide industrial applications due to its ability to hydrolyze complex proteins into simpler peptides and amino acids [2]. Owing to its versatility, papain is extensively utilized in the food, pharmaceutical, cosmetic, leather, and textile industries. In medicine, it is used in wound healing, digestive formulations, and anti-inflammatory preparations, while in the food industry it serves as a meat tenderizer and clarifying agent [7, 18].

The enzyme is synthesized and stored in specialized latex-producing cells known as laticifers and is most abundant during the early stages of fruit development [10]. At this stage, papain plays a crucial defensive role in the plant by degrading proteins of invading herbivores and pathogens [9]. Enzyme content and activity are highest a few weeks after fruit initiation, making this optimal period for latex tapping and papain extraction. Papain is obtained by making shallow incisions on unripe papaya fruits and collecting the latex that exudes from the surface, which is then dried and processed. As the fruit matures and ripens, papain content gradually declines due to reduced latex duct activity and changes in metabolic processes [3].

The synthesis of papain in *Carica papaya* is influenced by environmental conditions and crop management practices, which together determine both quantitative yield and qualitative stability of the enzyme. Temperature of 25–35 °C is favourable for maximum papain accumulation; however exposure to excessively high temperature may destabilize the enzyme, while low temperature suppress metabolic processes and restrict laticifer development. Relative humidity also plays an important role, as higher humidity facilitates continuous latex secretion, whereas well drained, fertile soil with ideal pH range is also essential for papain activity.

For effective utilization of papain in various applications, it is important to understand the behavior of the solution, particularly with respect to concentration. Among the available analytical techniques, spectrophotometric analysis is used for studying proteins due to its simplicity and rapid response. This method allows evaluation of enzyme characteristics based on absorbance at specific wavelengths, which is related to protein content [20].

In addition, optimization techniques play an important role in improving the application efficiency of papain in formulation-based systems. By using statistical tools such as Response Surface Methodology (RSM), critical parameters can be systematically evaluated to obtain stable and efficient formulations. Optimization helps in identifying the best combination of formulation variables to enhance stability, functionality, and performance in nano-edible coating systems [16, 13]. Despite the wide applications of papain, limited studies have focused on its spectrophotometric characterization across different concentrations and its integration into nano-edible coating systems using optimization techniques.

Therefore, the study was undertaken to extract papain from immature *Carica papaya*, analyze its absorbance behavior using spectrophotometric methods, and optimize papain-based nano-edible coatings using RSM for postharvest applications.

2. MATERIALS AND METHODS

2.1 Extraction of papain from papaya

Papain was extracted from immature fruits of *Carica papaya* through latex collection. The extraction process was conducted during the early morning hours under conditions of high humidity to facilitate maximum latex exudation [3]. Two to three longitudinal incisions were carefully made on the fruit surface using a sterile stainless-steel knife. The depth of each incision was maintained between 1–2 mm to ensure efficient latex release without causing excessive damage to internal tissues. The cuts must be scraped carefully and over a period of 12–16 days, this practice of creating four incisions on the untapped fruit surface at 3–4 day intervals is performed three or four times [21]. A milky fluid known as latex was collected in a plastic bowl until it stopped flowing. The latex is then transferred to an amber-coloured glass bottle to protect it from direct sunlight and prevent photo-degradation of enzymatic components. Foreign matters was removed by passing the latex through a sieve. To enhance stability and prolong shelf life, the filtered latex was treated with preservatives. Potassium Meta-bi sulfate (KMS)

0.05% and Benzoic acid 0.1% were added to papain to inhibit microbial growth, reduce oxidation, and maintain the stability and shelf life of the papain enzyme [2]. Papain is then dried using a tray dryer at 50–55°C for 24 h. Dried material of papain were obtained, which we powdered it using a pestle and mortar. The powdered papain is then packed in an air tight container and stored in a cool, dry place.

2.2 Preparation of stock solution and working solutions

Papain is widely regarded as safe for use in food systems and is permitted under Good Manufacturing Practices (GMP) as a food additive. According to the EFSA Panel on Food Enzyme [6], it is considered edible and non-toxic at recommended levels of use and in food and edible coating applications, papain is generally used at low concentrations, typically ranging from 0.05% to 1%, to ensure effectiveness without causing undesirable tissue degradation or sensory changes [10].

A stock solution of papain was prepared by dissolving 50 mg (0.05 g) of dried papain powder in 50 mL of lukewarm distilled water. The solution was stirred using a magnetic stirrer at 800–900 rpm to ensure complete and uniform dissolution of the enzyme, thereby avoiding the formation of aggregates or undissolved particles. From the prepared stock solution, a series of working concentrations of papain (10, 20, 30, 40, 50 and 60 $\mu\text{g mL}^{-1}$) were formulated by appropriate dilution of the stock solution using distilled water, following standard dilution principles [8, 17]. All dilutions were carried out using calibrated volumetric apparatus to ensure accuracy and consistency. The prepared solutions were gently mixed to maintain uniform distribution of papain molecules across all concentrations. Fresh preparations were used for each measurement to preserve from enzymatic integrity and to protected from direct light exposure until further use.

2.3 Determination of absorbance and selection of analytical wavelength

The absorbance of papain solutions was measured using a UV-Visible spectrophotometer, which covers both ultraviolet and visible regions and is suitable for protein analysis [4, 17]. During the analysis, samples were scanned over wavelength range of 200–800 nm to determine the wavelength of maximum absorbance (λ_{max}). The λ_{max} represents the point of highest absorption by the enzyme and reflects the characteristic absorption behavior of protein molecules. The wavelength having maximum absorbance was selected as the analytical wavelength and was used for comparison of absorbance values across different concentrations. This ensures consistency and accuracy in measurements. Using a fixed wavelength also minimizes variability and allow a reliable assessment of the relationship between enzyme concentration and absorbance.

Selection of the analytical wavelength helps in obtaining clear and accurate absorbance readings of papain. Measuring at the maximum absorbance wavelength improves sensitivity and ensures consistent results across all samples. It also reduces measurement errors and helps in reliable comparison of enzyme concentration in different solutions.

2.4 Formulation and analysis of coating

The development of papain-based nano edible coating was done using RSM (Response Surface Methodology) with Design of Experiment (DOE) software. RSM is a statistical and mathematical tool used to study the relationship between multiple formulation variables and their responses. It helps in understanding interaction effects between components and in identifying optimal conditions for desired outcomes. A Central Composite Design (CCD) was employed under RSM to evaluate the combined effects of formulation variables and optimize the coating composition [15, 14, 16].

A total of 20 experimental formulations were generated using CCD. The independent variables included oil, surfactant, and co-surfactant. The selected ranges oil (0.09–0.33%), surfactant (0.45–0.78 μg), and co-surfactant (0.09–0.33 μg), which were determined based on preliminary screening experiments and supported by literature on nano-emulsion formation and optimization [12, 11]. Batches were prepared using CCD (Central composite design).

Certain formulation parameters were kept constant for all experimental runs, including plasticizer (0.1) %, papain (150 μL), and water (25mL). These components were selected to ensure consistency and compatibility among different formulations.

We combined all the Variable factors- oil, surfactant, co-surfactant, given by CCD in range with all constant factors to form all the 20 batches. Each batch was stirred for 10 min in a magnetic stirrer to ensure uniform mixing. Subsequently, the formulations were subjected to ultrasonication using a Bath Sonicator for 1 hour at 50°C. The sonication process facilitates the formation of the stable nano-emulsions by reducing droplet size and enhancing dispersion [11].

2.5 Characterization of nano-coatings

The prepared nano-coating formulations were characterized based on particle size and PDI (Polydispersity Index) using a particle size analyser. The polydispersity index indicates the uniformity of the particle sizes within the formulation whereas particle size indicates how small the particles are, which affects surface area, activity, and effectiveness of the nano-formulation [5]. For each batch, the Particle size and PDI were calculated in CIF-LPU (Central Instrumentation Facility) and an analysis report was generated showing the quality result of the coating. The formulations exhibit particle sizes and PDI values were considered more stable and suitable for further application. The selected data were incorporated in central composite design table for further analysis.

2.6 Statistical Analysis

The experimental result were processed using Design of experiment (DOE) software to examine the effects of formulation variables on the response parameters. Analysis of Variance (ANOVA) was applied to determine the significance of the independent variables and their interactions on the response parameters.

Model accuracy was determined based on F-value and corresponding p-value at a 95% confidence level ($p < 0.05$), with terms considered statistically significant when the probability of error was less than 5%.

The model was further evaluated using adjusted and predicted values to ensure good agreement between experimental and predicted results. Additionally, lack of fit test was performed to verify the suitability of the model. Overall, the statistical analysis confirmed the reliability and validity of the experimental results.

3. RESULTS AND DISCUSSION

3.1 Spectrophotometric Analysis of Papain

The absorbance of papain at different concentrations level were measured using spectrophotometric analysis, and the results are presented in Table 1 and Fig. 1. The absorbance values were recorded within the wavelength range of 200–800 nm. The papain showed maximum absorbance in the ultraviolet region, ranging from (274 to 277 nm) across the different concentrations. This is characteristic of protein molecules, as aromatic amino acids present in enzymes generally exhibit strong UV absorption near 280 nm [24].

Table 1 shows a gradual increase in absorbance with increasing concentration of papain from 10 to 50 $\mu\text{g mL}^{-1}$ where, the absorbance values increased from 0.025 at 10 $\mu\text{g mL}^{-1}$ to a maximum value of 0.125 at 50 $\mu\text{g mL}^{-1}$. This trend follows the Beer-Lambert law, indicating a direct proportional relationship between concentration and absorbance within the range. However, at a higher concentration of 60 $\mu\text{g mL}^{-1}$, a slight decrease in absorbance (0.120) was recorded, suggesting a deviation from linearity at higher concentrations, possibly due to protein aggregation or light scattering effects [19]. Therefore, the concentration ranging from 10–50 $\mu\text{g mL}^{-1}$ was found to be optimal for reliable spectrophotometric estimation of papain.

For consistency in comparison, the wavelength of 277 nm was considered for further analysis, and the corresponding results are presented in Table 2 and Fig. 2.

The linearity observed within the optimal concentration range helps in accurate measurement of papain during formulation and analysis. This is important to maintain consistency in nano-edible coating formulations.

This result is useful for developing nano-edible coating development, accurate measurement of papain helps control its enzymatic activity in the coating system. The relationship between absorbance and concentration helps in better formulation control, ensuring the correct amount of papain being added to the nano-emulsion system. This improves coating uniformity, stability, and overall efficiency.

Overall, the spectrophotometric analysis shows that papain can be measured accurately within a certain concentration range. This supports its effective use in nano-edible coatings and helps improve the quality, consistency, and performance of coatings in post-harvest applications.

Table 1
Absorbance of papain at different wavelength

Concentration ($\mu\text{g/mL}^{-1}$)	Wavelength(nm)	Absorbance
10	274	0.025
20	276	0.049
30	277	0.078
40	277	0.103
50	277	0.125
60	277	0.120

Table 2
Absorbance of papain at fixed wavelength (277nm)

Concentration ($\mu\text{g/mL}^{-1}$)	Wavelength(nm)	Absorbance
10	277	0.021
20	277	0.035
30	277	0.078
40	277	0.103
50	277	0.125

3.2 Optimization and analysis of nano-edible coating

The formulations of papain-based nano-edible coatings was optimized using Response Surface Methodology (RSM) based on a Central Composite Design (CCD) [14]. A total of 20 experimental combinations were generated to examine how the influence of independent variable, namely oil (A), surfactant (B), and co-surfactant (C), on key response parameters such as particle size and polydispersity index (PDI). The design framework allowed evaluation of both individual and interactive effects of formulation components on nano-emulsion characteristics [15]. Statistical analysis of the experimental data was performed

Particle Size

The Analysis of Variance (ANOVA) for particle size indicated that the selected quadratic model was highly significant, with an F-value of 45.19 and a very low probability level ($p = < 0.0001$). This suggests that the model was adequate for predicting and explaining the relationship between formulation variables and response outcomes. Among the main factors, oil (A) and co-surfactant (C) showed significant effects on particle size, while surfactant (B) alone did not exhibit a statistically significant influence. However, interaction effects were found to be highly significant, particularly between oil and

co-surfactant (AC) and surfactant and co-surfactant (BC), with F-values of 53.14 and 69.19, respectively. These results highlight the critical role of combined interactions, especially involving the co-surfactant, in determining the efficiency of nano-emulsion formation [23].

The lack-of-fit test was not significant (p = 0.4622), indicating that the model fits the experimental data well and can reliably predict responses within the studied range [15].

The model exhibited high coefficient of determination ($R^2 = 0.9760$), showing that 97.60% of the variability in particle size. The adjusted R^2 (0.9544) and predicted R^2 (0.8866) were in reasonable, confirming good predictive system behavior. In addition, the adequate precision value (27.453), representing the signal-to-noise ratio, was significantly greater than the desirable value of 4, indicating an adequate signal and confirming the model reliability.

Table 3 ANOVA of Particle size						
Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	73801.75	9	8200.19	45.19	< 0.0001	significant
A-Oil	13204.05	1	13204.05	72.76	< 0.0001	
B-Surfactant	223.48	1	223.48	1.23	0.2931	
C-Co- Surfactant	4093.43	1	4093.43	22.56	0.0008	
AB	206.55	1	206.55	1.14	0.3111	
AC	9643.13	1	9643.13	53.14	< 0.0001	
BC	12557.16	1	12557.16	69.19	< 0.0001	
A ²	16585.43	1	16585.43	91.39	< 0.0001	
B ²	1198.85	1	1198.85	6.61	0.0279	
C ²	12755.39	1	12755.39	70.29	< 0.0001	
Residual	1814.77	10	181.48			
Lack of Fit	947.83	5	189.57	1.09	0.4622	not significant
Pure Error	866.93	5	173.39			
Cor Total	75616.52	19				

Polydispersity Index (PDI)

Analysis of Variance (ANOVA) for polydispersity index (PDI) indicated that the developed quadratic model was highly significant, with an F-value of 14.04 and probability level of p = 0.0001. This confirms

that the formulation was adequate for describing the relationship between formulation variables and PDI of the nano-emulsion system.

The lack-of-fit test was non-significant ($F = 0.2076$, $p = 0.9453$), confirming that the model adequately fitted the experimental data and that the unexplained variation was minimal. Therefore, the model can be considered reliable for prediction and optimization of PDI within the selected experimental range [15].

The model exhibited a high coefficient of determination ($R^2 = 0.9267$), indicating a good fit between experimental and predicted values. The adjusted R^2 (0.8607) and predicted R^2 (0.8138) were in close agreement, confirming acceptable predictive performance of the model. The adequate precision value (13.716), greater than the desirable value of 4, indicating an adequate signal and confirming the model reliability.

Table 4
ANOVA of Polydispersity Index

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0312	9	0.0035	14.04	0.0001	significant
A-Oil	0.0010	1	0.0010	3.89	0.0769	
B-Surfactant	0.0016	1	0.0016	6.62	0.0278	
C-Co- Surfactant	0.0072	1	0.0072	29.04	0.0003	
AB	0.0001	1	0.0001	0.2920	0.6008	
AC	0.0087	1	0.0087	35.33	0.0001	
BC	0.0033	1	0.0033	13.30	0.0045	
A ²	1.550E-06	1	1.550E-06	0.0063	0.9384	
B ²	0.0056	1	0.0056	22.85	0.0007	
C ²	0.0045	1	0.0045	18.21	0.0016	
Residual	0.0025	10	0.0002			
Lack of Fit	0.0004	5	0.0001	0.2076	0.9453	not significant
Pure Error	0.0020	5	0.0004			
Cor Total	0.0336	19				

3.3 Optimization Outcomes

Optimization analysis derscribed that the desirable formulation was obtained at suitable levels of oil, surfactant, and co-surfactant, giving particle size ranging from (205.4-466.6 nm) and PDI (0.30–0.45). This indicates a stable nano-emulsion with uniform droplet distribution. Higher surfactant levels helped

reduce interfacial tension and formed smaller, more stable droplets. The optimized ones showed better results than non-optimized formulations.

Analysis confirmed the formation of nano-scale droplets with good stability. The interaction among oil, surfactant, and co-surfactant played important role in proper emulsification. Smaller particle size increases surface area, which improves coating spread, adhesion, and barrier properties against moisture loss and gas exchange. Low PDI values indicate better uniformity and consistency of the coating. The optimized surfactant and co-surfactant combination also helped maintain nano-structure stability and may improve the activity of papain in the coating system.

Overall, RSM proved to be an effective method for optimization. The developed papain-based nano-edible coating has potential for improving stability and extending shelf life of fruits during storage.

4. CONCLUSION

The present study demonstrated the successful extraction and characterization of papain from immature *Carica papaya* latex and its application in nano-edible coating systems. Spectrophotometric analysis determined maximum absorbance in the ultraviolet region (274–277 nm), with a linear relationship between concentration and absorbance observed within 10–50 $\mu\text{g mL}^{-1}$, confirming the suitability of this range for accurate estimation of papain. Optimization of the nano-edible coating using Response Surface Methodology (RSM) revealed that formulation variables significantly influenced particle size and polydispersity index (PDI). The optimized formulation exhibited desirable particle size showing good stability and uniformity. Overall, the results indicate that papain-based nano-edible coatings have potential for improving formulation stability, maintaining enzymatic activity, and enhancing postharvest quality and shelf life of fruits. This study supports the application of enzyme-based nano-coatings as an effective approach for postharvest preservation.

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Figures

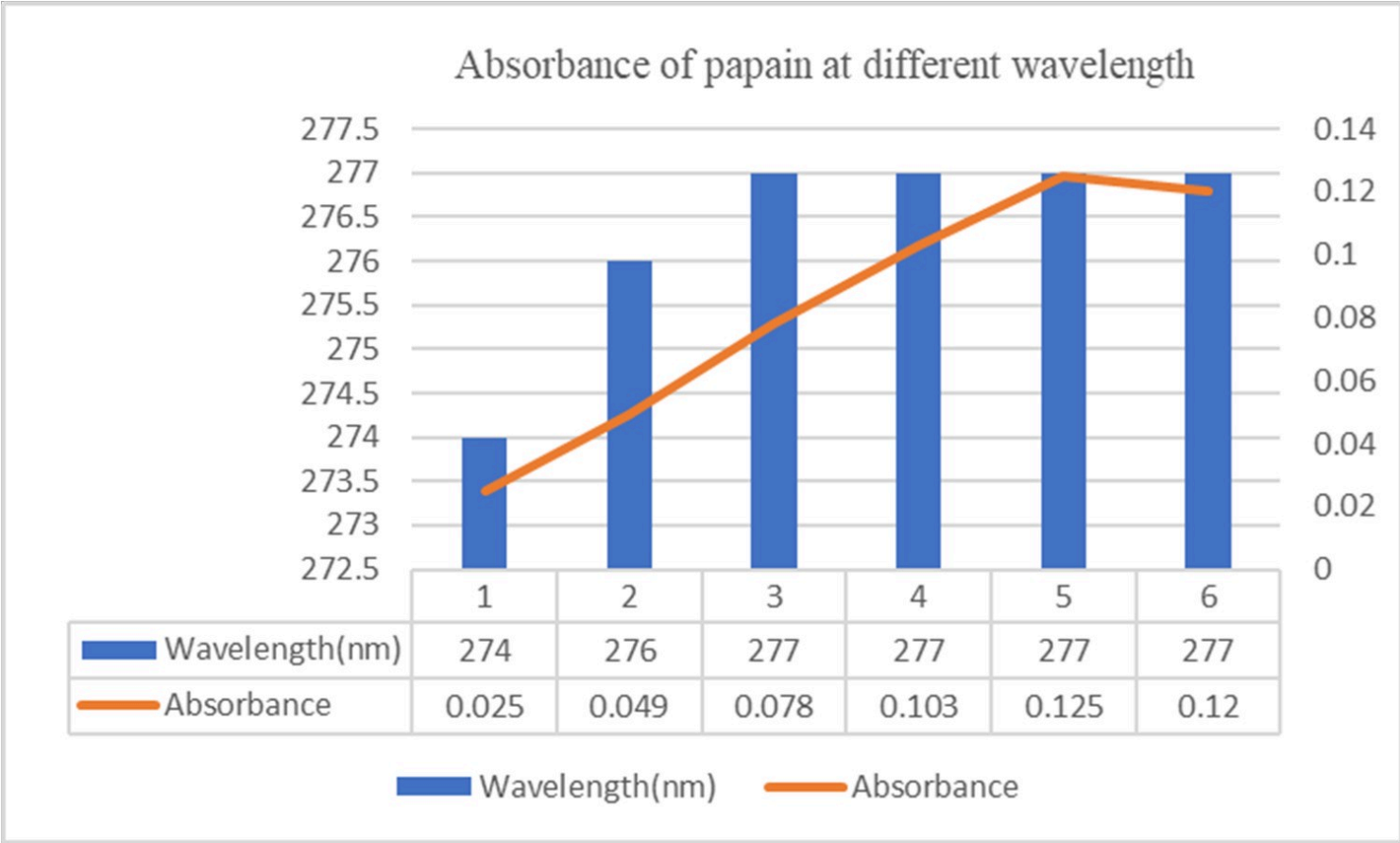


Figure 1

Absorbance of papain at different wavelength

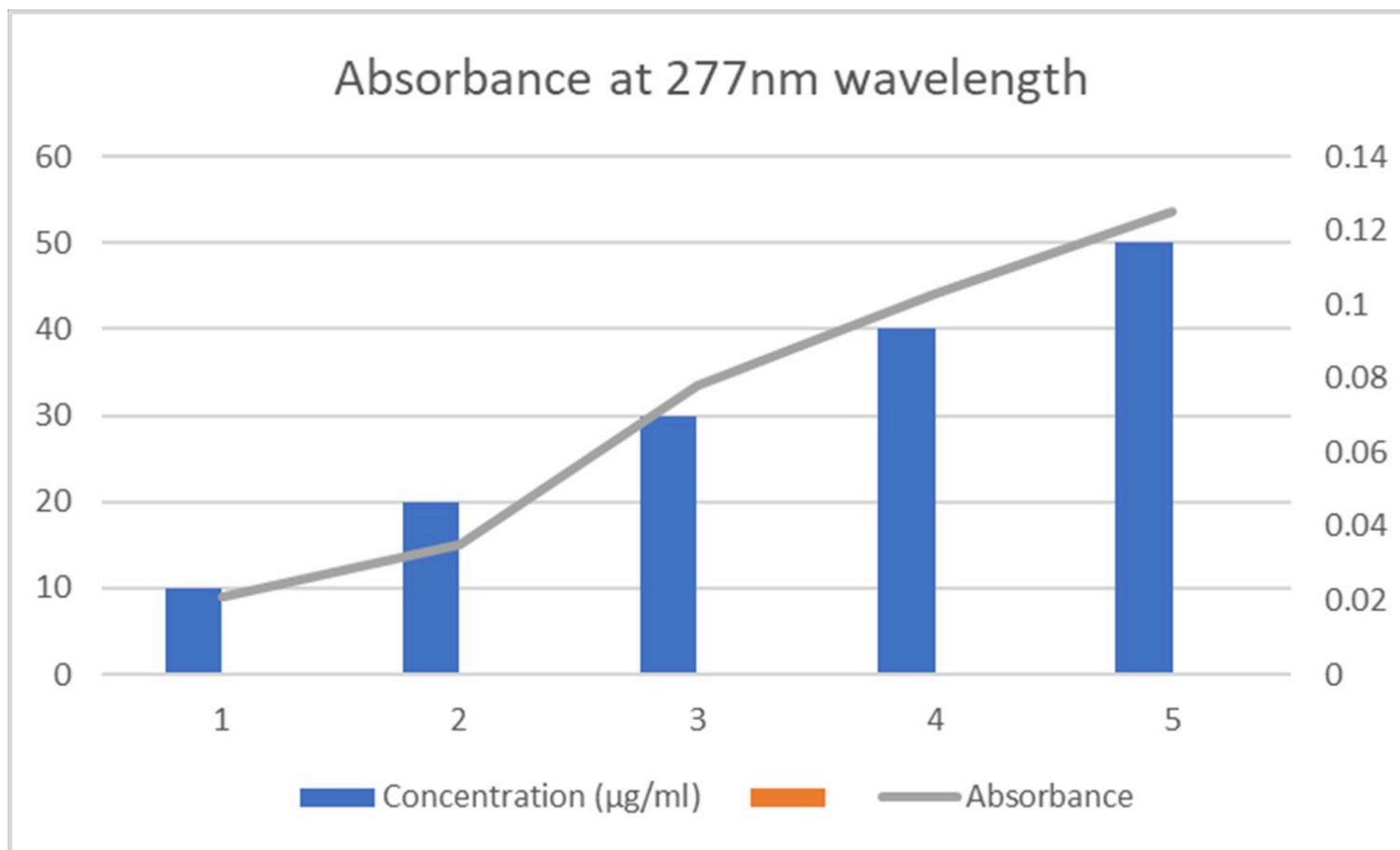


Figure 2

Graph of fixed wavelength (277nm)