Development of UV-Vis Spectroscopic Method for Determining Effect of Solvent on Solubility of Vitamin C and Vitamin E

By

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis/project titled “Development of UV-Vis Spectroscopic method for determining effect of solvent on solubility of vitamin C and vitamin E” submitted by Raisa Thasnim Anni (16146027) of Spring 2016 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on 27th January 2020.

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Ethics Statement

The study does not involve any kind of animal or human trial.

Abstract

This study was done to observe effect of solvent on solubility of vitamins. Vitamins studied included vitamin C (water soluble) and vitamin E (fat soluble). The solvents used included acetonitrile and distilled water. The proportions of acetonitrile and water in the solvent were varied and the effects on solubility of the vitamins were determined. To determine the solubility, UV-Vis spectroscopy methods were developed. These methods were also validated. The result that found from experiment was represented on calibration curve and discussed if any error found. As multi vitamin carries both water soluble and fat soluble compound, this study was carried out to find whether they showed same effect or not. This will be useful for conducting experiments in the future and also these types of comparative study will enrich the performance of quantitative analysis.

Keywords: UV-Vis spectroscopy; Method development; Method validation; Vitamins; solubility.

Dedication

Dedicated to my parents

Acknowledgement

On the accomplishment of my project, I would like to thank the Almighty Allah blessing me with patience to complete this project. My journey with this project was a learning experience for me and I could come this far because of the constant support of some people who have been thoughtful with this project.

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List of Acronyms

UV-VIS	Ultraviolet-visible
RSD	Relative Standard Deviation

Glossary

A_1^1	This term refers to the to the absorbance of 1 cm layer of the solution whose concentration is 1 % at a specified wavelength.
Dissolution	Dissolution is a process in which solid substances solubilizes in a given solvent.
Endothermic	Endothermic is a process which requires or absorbs energy from its surroundings.
Equilibrium	A state in which the rate of the forward reaction equals the rate of the backward reaction.
Exothermic	An exothermic is a process which releases energy.
Hydrogen bond	A weak bond between two molecules resulting from an electrostatic attraction.
Hydrogen bond acceptor	The hydrogen acceptor is the neighboring electronegative ion or molecule, which has a lone electron pair in order to form a hydrogen bond.
Hydrogen bond donor	It can be defined as the atom to which the hydrogen atom participating in the hydrogen bond is covalently bonded.
Le Chatelier's principle	It states that changes in the temperature, pressure, volume, or concentration of a system will result in predictable and opposing changes in the system in order to achieve a new equilibrium state.
Linearity	The linearity of an analytical method can be explained as its capability to show "results that are directly

	proportional to the concentration of the analyte in the sample.
Log P	A partition coefficient or distribution coefficient is the ratio of concentrations of a compound in a mixture of two immiscible solvents at equilibrium. This term is the log of partition coefficient.
Method validation	It is the process to confirm that the analytical procedures are designed for specific test is suitable for use.
Molar absorptivity	The property that measures the strength by which a chemical species absorbs light at a particular wavelength.
%v/v	Volume concentration of solution which defines as the volume in mL per 100 mL of solution.
%w/v	Mass concentration of solution which defines weight in g per 100 mL of solution.
Repeatability	Repeatability is the closeness of agreement between mutually independent test result obtained with the same method on identical test material in the same laboratory by the same analyst using the same equipment with in short interval of time.
RSD	It is known as the coefficient of variance. The RSD measures the precision of the average of results.
R^2	It is a statistical that measure of how close the data are to the fitted regression line.
Saturated solution	Solution that contains the maximum concentration of a

	solute that can be dissolved in the solvent.
Solute	Small amount of substance that dissolve in solution.
Solvent	Substance in which solute is dissolved to form a solution.
Van der Waals force.	A term used to define the attraction of intermolecular forces between molecules.

Chapter 1

Introduction

1.1 Factors affecting absorbance in UV-Vis Spectroscopy

Spectroscopy can be defined as "the interaction between matter and electromagnetic radiation." One type of spectroscopy is ultraviolet spectroscopy, which is widely used in the quantitative analysis of organic compounds. When, ultra violet or visible light is passed through an organic compound, electronic transition may take place(Guthrie, 1979). A UV-Vis spectrophotometer consists of a light or radiation source, monochromator, sample compartment, detector, amplifier and a recorder. The UV-visible absorption spectrum of a can be obtained by plotting absorbance over the wavelength range 200-800nm. Here, absorbance can be defined as the ratio of intensity of light incident upon the sample cell and the intensity of light leaving the sample cell. The absorbance of a sample depends on several factors. This essay summarizes some of those factors.

To begin with, the absorbance of a sample solution of a compound is directly proportional to its concentration. From Beer-Lambert Law, we can see that, $A= \varepsilon cl$. Where, A is absorbance, c is molar concentration of solute, l is length of sample cell in centimeters and ε molar absorptivity (Guthrie, 1979). The higher the concentration of solute, the higher will be the absorbance. The lower the concentration of solute, the lower will be the absorbance. This relationship can be used to measure the unknown concentration of a compound in a solution. However, errors in measurement of concentration are minimum when the absorbance in the range 0.3 to 1.5 (Beckett et al., n.d.). The Beer-Lambert is not followed outside this range. For reliable results, in any assay the concentration should be adjusted so that measured absorbance in this range.

A compound absorbs radiation depending on its structure. Electronic transition only occurs when radiation of certain wavelength is passed through the sample. The value of this wavelength depends on the structure of the compound, more specifically chromophore and auxochrome. Chromophore can be defined as any isolated covalently bonded group that shows a characteristic absorption in the ultraviolet and visible light. Auxochromes can be defined as any group which does not itself act as a chromophore but whose presence brings about a shift of the absorption band towards (Guthrie, 1979). For example, benzene is a chromophore which shows a maximum absorption at 255nm. Lambda max is the wavelength where maximum absorption occurs.

The solvent used to make the sample solution can also affect the absorbance of the sample. If the solvent itself absorbs at the selected wavelength during measurement, the measure absorbance will not result from the solute alone. Therefore, when choosing a solvent to prepare sample solution, a solvent transparent at the selected wavelength should be chosen. The pH of the solvent plays a significant role in absorbance when the solute is ionizable (Guthrie, 1979).

To conclude, it is clear that to obtain a good UV-Vis spectrum, concentration, wavelength selection and choice of solvent should be appropriate.

1.2 Solubility

Solubility is “the concentration of solute in a saturated solution at a certain temperature”, or “the spontaneous interaction of two or more substances to form a homogeneous molecular dispersion” (Sinko, 1993) . This essay will explores which affect solubility.

The solubility of substances depends on various factors such as temperature of solvent, polarity of solvent etc. Polarity of the solvent has a great influence in solubility. Polar

solvent like water can dissolve salts and compound with dipole moments. Solubility of compounds in water increases if the solute molecule can form hydrogen bond with water molecule. Non polar solvents such as hydrocarbon do not form hydrogen bonds and cannot ionize salts. Therefore, they poorly dissolve polar substances. However, they dissolve non polar solutes by interacting by weak van der Waals forces.

Temperature can affect the solubility. If a dissolution process is endothermic, the temperature of the system will decrease upon dissolution. According to Le Chatelier's principle, providing heat will increase dissolution and therefore increase solubility. On the other hand, for exothermic dissolution process increase of temperature will decrease solubility. (Aulton, 2013). Therefore, it is important to maintain constant temperature when solubility of a substance is determined.

Thermodynamic solubility of a substance in a solvent is defined as “the maximum amount of the most stable crystalline form that remains in solution in a given volume of the solvent in a solution in a given volume of the solvent at a temperature and pressure under equilibrium conditions.” Thermodynamic solubility of a substance can be determined by adding the substance to a specific amount of solvent until there is no more visible dissolution. The system is then allowed to reach equilibrium. The excess substance is then removed and the concentration of the solution is then measured. This value describes the thermodynamic solubility of a substance. Determining thermodynamic solubility using the shake flask method is the most common approach in determining solubility.

In this project, the thermodynamic solubility of two vitamins will be determined using the shake flask method.

1.3 Vitamins

Vitamins were first discovered in Europe in the early 1900s. Since then, there have been many studies on them. Vitamins are essential organic compound, which regulates body growth, immunity and many other important activities. (Eggersdorfer et al., 2012) .Vitamins cannot be synthesized in humans and animals. As a result, vitamins should in our regular diet. Diets which are deficient in vitamins can be supplement by vitamins from non-food sources such as tablet.

Vitamins can be classified as fat-soluble and water-soluble. The fat soluble vitamins include vitamin A,D,E and K. The water soluble vitamins include vitamins C,B_1,B_2,B_3,B_5,B_6,B_7,B_9, and B_{12}. In this project, vitamin C and vitamin E were studied. The physicochemical properties (DL-alpha-Tocopherol acetate | C31H52O3 - PubChem, n.d.) (Ascorbic acid | HC6H7O6 - PubChem, n.d.) and uses (Irfan et al., 2017) (Muzaffar Ali Khan Khattak, n.d.) of these vitamins have been summarized in the following tables.

Table 1: Physicochemical properties of test vitamins

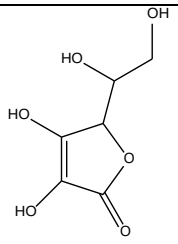
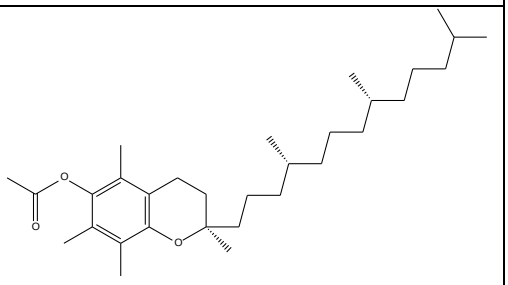
Vitamin	Ascorbic acid	Alpha tocopherol acetate
Structure		
Log P	-1.6	10.8
Hydrogen bond donor	4	0
Hydrogen bond acceptor	6	3

Table 2: Uses of tested vitamins

Vitamin	Uses
Vitamin C (Ascorbic acid)	• Reduces cholesterol in blood • Aids in absorption of iron. • Treats common cold. • Participate in formation and maintenance of collagen. • Helps in wound healing. • Acts as an antioxidant.
Vitamin E (Alpha tocopherol acetate)	• As an immune-regulator, it plays an important role in enhancing immune response. • Used in treatment of Ataxia. • High amount of vitamin E can reduce the risk of coronary heart disease. • It prevents the early stage of arthrosclerosis.

1.4 Aims

The aims of this study are:

- To develop analytical method based on UV-Vis spectroscopy for the determination of two vitamins in solvents with varying polarity.
- To validate the developed methods.

- To determine solubility of vitamin E and vitamin C in pure acetonitrile and pure water respectively, and mixtures of these two solvents.

Chapter 2

Materials

Materials that were used in this project are given below:

Table 3: Details of reagents

Reagents	CAS No	Company Name	Country of origin
Vitamin C	50-	Sisco Research Laboratories Pvt. Ltd	India
Vitamin E	7695-91-2	Loba Chemie Pvt. Ltd	India
Acetonitrile	75-05-08	Active Fine Chemicals Ltd	Bangladesh
Distilled water	-	BOE	Germany

Instruments that were used in this project are given below:

Table 4 Details of instruments

Instruments	Model no.	Company no
UV spectrophotometer	UV-1800	SHIMADZU
Distilled water plant.	BOE-8704000	BOECO
Shaking incubator	I10-OE+OL30-ME	OVAN
Centrifuge machine	80-2	WINCOM COMPANY LTD.
Vortex	MX-S	D-LAB
Weighing machine	FGH	A&D COMPANY LTD.

Chapter 3

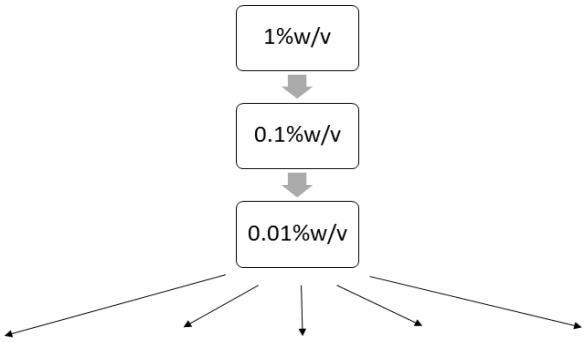
Methods

3.1 Determination of lambda max of Vitamin C

A 1% w/v solution of vitamin C was prepared in a 10 ml volumetric flask. Distilled water was used as the solvent. Vitamin C was weighed by difference. Calculations of weights are shown in the appendix.

The weighed amount was taken in the volumetric flask. Sufficient water was added to make up to 10ml. Absorbance of this solution was taken using distilled water as blank. The solution was diluted until absorbance was within 0.5 - 1.5. Dilution was performed using distilled water as solvent.

Next, the effect of adding acetonitrile in the solvent on the lambda max was tested. The 1% w/v solution was diluted again with varying volumes of distilled water and acetonitrile as shown in figure below:



%v/v acetonitrile	0	5	10	20	40
Volume of 0.01%w/v vitamin C solution (mL)	2	2	2	2	2
Volume of acetonitrile (mL)	0	0.5	1	2	4
Volume of distilled water (mL)	8	7.5	7	6	4
Total volume (mL)	10	10	10	10	10

Figure 1: Preparation of solutions of vitamin C for determining lambda max

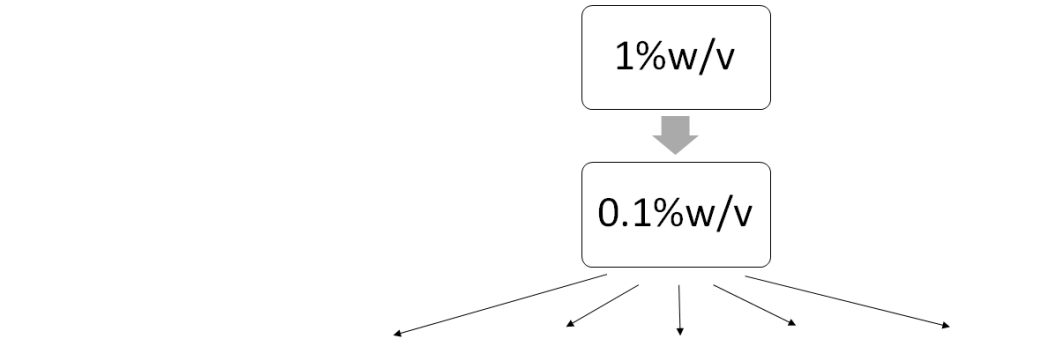
For each solution, the absorbance was measured over the range 200 nm – 400 nm. Lambda max and the absorbance at the lambda max for each solution was compared. The average of these values was calculated and used in subsequent experiments.

3.2 Determination of lambda max of vitamin E

A 1% w/v solution of vitamin E was prepared in a 10 ml volumetric flask. Acetonitrile was used as the solvent. Vitamin E was weighed by difference. Calculations of weights are in the appendix.

The weighed amount was taken in the volumetric flask. Sufficient acetonitrile was added to make up to 10ml. Absorbance of this solution was taken using acetonitrile as blank. The solution was diluted until absorbance was within 0.5 - 1.5. Dilution was performed using acetonitrile as solvent.

Next, the effect of adding water in the solvent on the lambda max was tested. The 1%w/v solution was diluted again with varying volumes of acetonitrile and distilled water as shown in figure below:



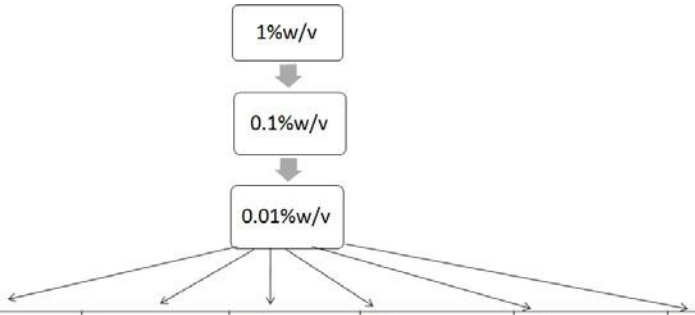
%v/v distilled water	0	5	10	20	40
Volume of 0.1%w/v vitamin E solution (mL)	2	2	2	2	2
Volume of distilled water(mL)	0	0.5	1	2	4
Volume of acetonitrile (mL)	8	7.5	7	6	4
Total volume (mL)	10	10	10	10	10

Figure 2 : Preparation of solutions of vitamin E for determining lambda max

For each solution, absorbance was measured over the range 200 nm – 400 nm. Lambda max and the absorbance at the lambda max for each solution was compared. The average of these values was calculated and used in subsequent experiments.

3.3 Method validation for vitamin C

To construct a calibration curve, a 1%w/v stock solution was diluted using distilled water to six different concentrations. The dilutions performed are shown in the figure below:



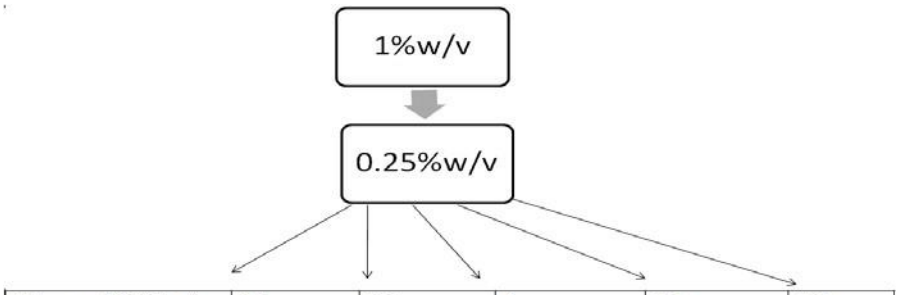
Volume of 0.01% w/v vitamin C in solution (ml)	0.8	1	1.1	1.2	1.3	1.4
Final volume (ml)	10	10	10	10	10	10
Final concentration (%w/v)	0.001	0.0013	0.0014	0.0015	0.0017	0.002

Figure 3: Dilution of 1%w/v vitamin C for construction of calibration curve

The absorbance for each solution was measured at the lambda max determined in experiment 3.1. The calibration curve was constructed using Microsoft Excel. The linearity was also assessed. To assess repeatability, the 1%w/v stock solution was diluted three times to the same concentration. The absorbance of the solutions was measured. The concentrations were calculated using the calibration curve. The relative standard deviation was calculated.

3.4 Method validation for vitamin E

To construct a calibration curve, a 1%w/v stock solution was diluted using acetonitrile to five different concentrations. The dilutions performed are shown in the figure below:



Volume of 0.25%w/v vitamin E solution (ml)	0.5	0.6	1	1.1	1.2
Final volume(ml)	10	10	10	10	10
Final concentration (%w/v)	0.0005	0.0006	0.001	0.0011	0.0012

Figure 4: Dilution of 1%w/v vitamin E solution for construction of calibration curve

The absorbance for each solution was measured at the lambda max determined in experiment 3.2. The calibration curve was constructed using Microsoft Excel. The linearity was also assessed. To assess repeatability, the 1%w/v stock solution was diluted three times to the same concentration. The absorbance of the solutions was measured. The concentrations were calculated using the calibration curve. The relative standard deviation was calculated.

3.5 Determination of solubility of Vitamin C in solvents of varying polarity

The solubility of vitamin C was determined in mixtures of distilled water and acetonitrile. First, the following solution was made in duplicates in 15ml falcon tubes.

Table 5 Preparation of solvents for determination of solubility of vitamin C

Solution	Volume of distilled water (mL)	Volume of acetonitrile (mL)	Total volume (mL)
0%v/v acetonitrile	5	0	5
10%v/v acetonitrile	4.5	0.5	5

20%v/v acetonitrile	4	1	5

Next, in each falcon tube, vitamin C was added and vortexed. If there was no precipitate visible, more vitamin C was added, and the solution was vortexed. This was continued until a precipitate appeared. All the falcon tube, were then shaken in the shaking incubator for 2 hours at rpm 280 at 25°C. After two hours, the solutions were centrifuged at rpm 1000 for 10 minutes. The absorbances of the supernatants were then measured. The supernatants were diluted with distilled water so that the absorbance remained within the range of the calibration curve. The absorbances of the final solutions were measured. These were used to calculate the concentration of the solutions using the calibration curve constructed in experiment 3.3.

3.6 Determination of solubility of Vitamin E in solvents of varying polarity

The solubility of vitamin E was determined in mixtures of distilled water and acetonitrile.

First, the following solution was made in duplicates in 15ml falcon tubes.

Table 6 Preparation of solvents for determination of solubility of vitamin E

Solution	Volume of acetonitrile (mL)	Volume of distilled water (mL)	Total volume (mL)
0 %v/v water	5	0	5
10%v/v water	4.5	0.5	5
20%v/v water	4	1	5

Next, in each falcon tube, vitamin E was added and vortexed. If the solution was clear, then more vitamin E was added and the solutions were vortexed. This continued until the solution

seemed cloudy. All the falcon tubes were then shaken in the shaking incubator for 2 hours at rpm 280 at 25°C. After two hours, the solutions were centrifuged at rpm 1000 for 10 minutes. The absorbances of the supernatants were then measured. The supernatants were diluted with acetonitrile so that the absorbance remained within the range of the calibration curve. The absorbances of the final solutions were measured. These were used to calculate the concentration of the solutions using the calibration curve constructed in experiment 3.4.

Chapter 4

Results

4.1 Determination of lambda max of vitamin C

The following spectrum was obtained for the different solutions of vitamin C. The absorbance at each peak is given in the appendix.

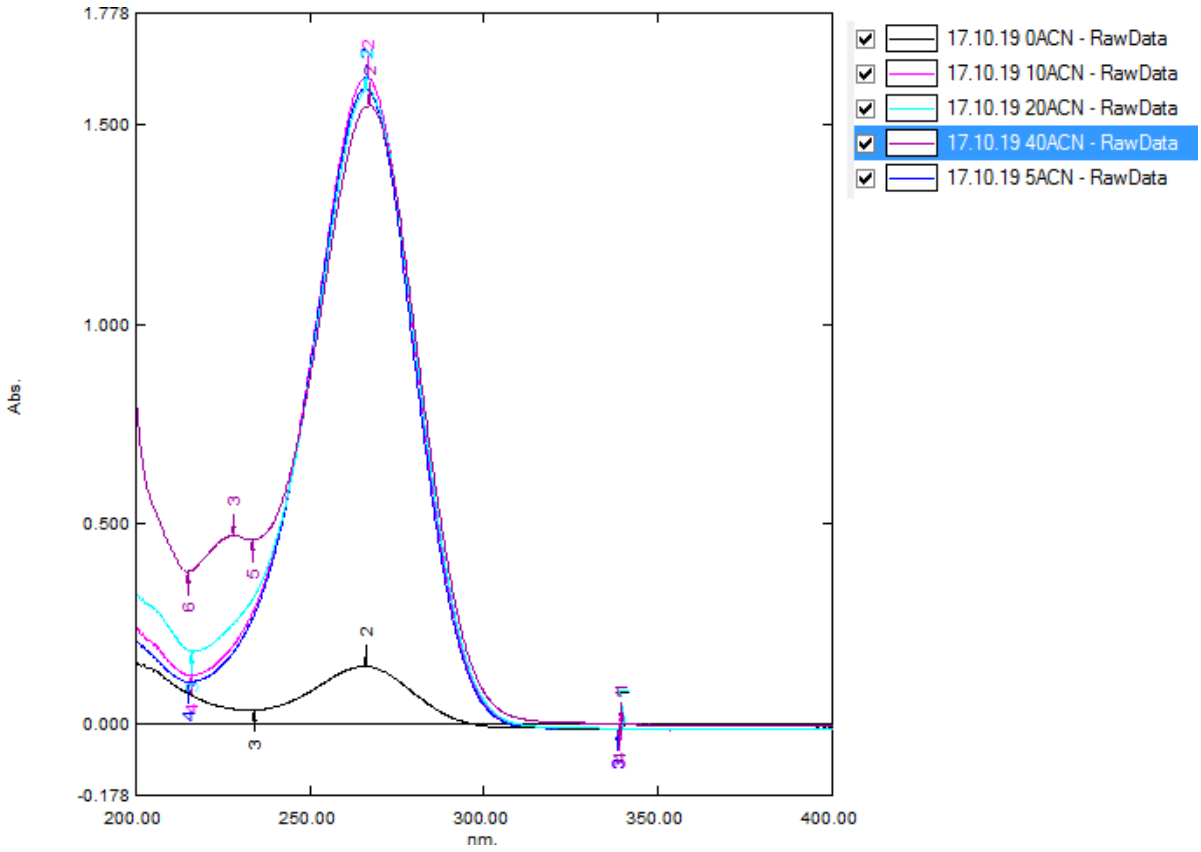


Figure 5: Spectra of vitamin C in different solvents

The value of absorbance found for vitamin C in 0% v/v acetonitrile was not considered because of high possibility of error. It is likely that the solution was not properly prepared.

The lambda max was calculated to be at 266 nm. The average absorbance at this wavelength was 1.58. Calculations are included in the appendix.

4.2 Determination of lambda max of vitamin E

The following spectrum was obtained for the different solutions of vitamin E**.** The absorbance at each peak is given in the appendix.

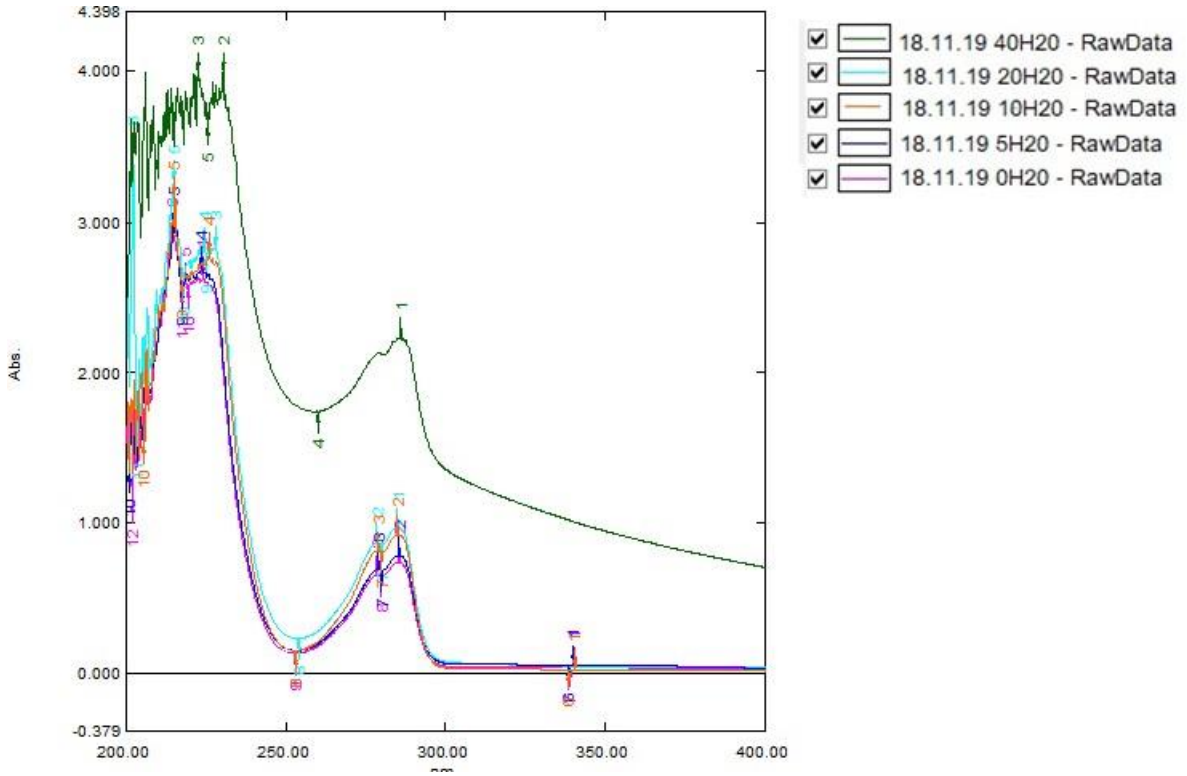


Figure 6 : Spectra of vitamin E in different solvents.

The value of absorbance found for vitamin E in 40% v/v water was not considered because of high possibility of error. This solution was visibly turbid.

The lambda max was calculated to be at 285nm. The average absorbance at this wavelength was 0.853. Calculations are included in the appendix.

4.3 Method validation for vitamin C

The absorbance of the solutions was measured. The concentrations of the solution were calculated using the weight of vitamin C used in preparing the stock solution. All calculations are shown in appendix. These values were used to construct a calibration curve.

Table 7 Measurements of absorbance for construction of calibration curve for vitamin C

Concentration (%w/v)	Absorbance
0.00111	0.826
0.001443	0.937
0.001554	0.993
0.001665	1.093
0.001887	1.181
0.00222	1.328

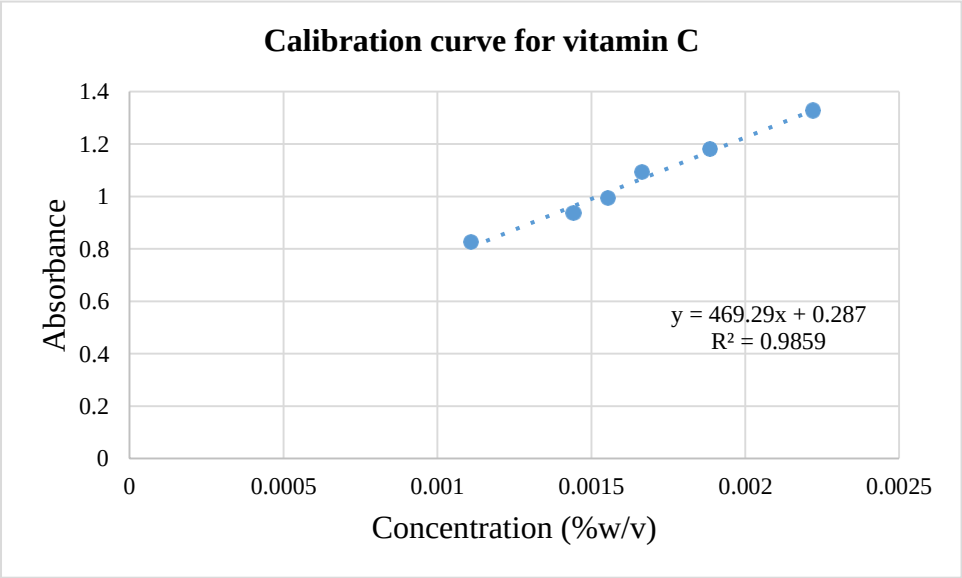


Figure 7: Calibration curve for vitamin C

The equation of the line was found to be y = 469.29x + 0.287 and R^2 was found to be 0.9859.

To assess repeatability, solutions of same concentration were prepared. The absorbance of these solutions was measured. The concentrations were calculated using calibration curve. The results are summarized below.

Table 8 Concentrations of replicates of vitamin C solution

Replicate	Absorbance	Concentration (%w/v)	Average concentration (%w/v)	RSD
1	1.093	0.001718		
2	1.072	0.001673	0.001741	4.736%
3	1.147	0.001833		

4.4 Method validation for vitamin E

The absorbance of the solutions was measured. The concentrations of the solution were calculated using the weight of vitamin E used in preparing the stock solution. All calculations are shown in appendix. These values were used to construct a calibration curve.

Table 9 Measurements of absorbance for construction of calibration curve for vitamin E

Concentration (%w/v)	Absorbance
0.013	0.5
0.0156	0.606
0.026	1.046
0.0286	1.145
0.0312	1.291

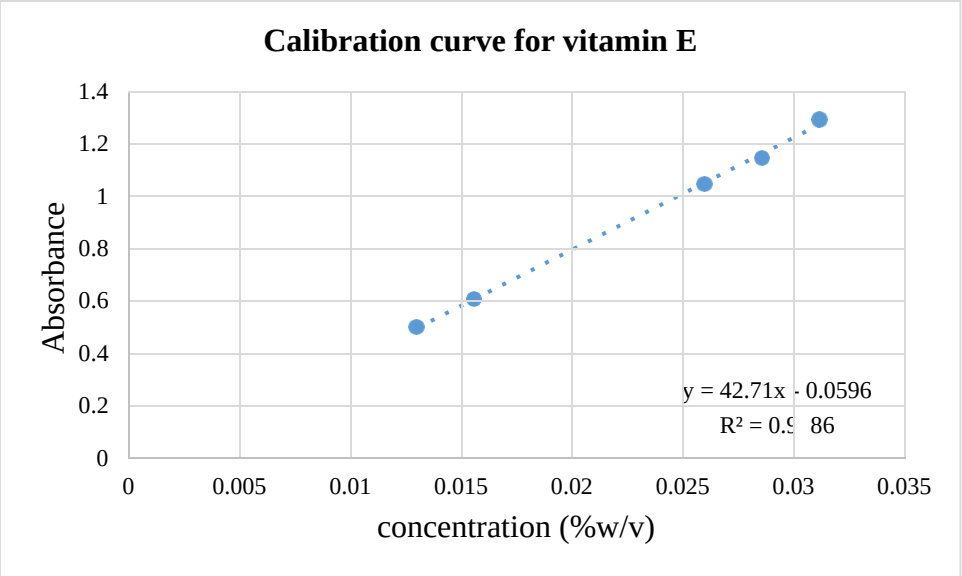


Figure 8: Calibration curve for vitamin E

The equation of the line was found to be y = 42.71x - 0.0596 and R^2 was found to be 0.9986.

To assess repeatability, solutions of same concentration were prepared. The absorbance of these solutions was measured. The concentrations were calculated using calibration curve. The results are summarized below.

Table 10 Concentrations of replicates of vitamin E solution

Replicate	Absorbance	Concentration (%w/v)	Average concentration (%w/v)	RSD
1	1.291	0.003162		
2	1.258	0.003085	0.003101	1.791%
3	1.245	0.003055		

4.5 Determination of solubility of Vitamin C in solvents of varying polarity

The calibration curve that was constructed in 4.3 was used to calculate the concentration of the following solutions. Example of calculation is shown in the appendix.

Table 11 Concentrations of vitamin C in different solvents

Concentration of acetonitrile (%v/v)	Replicate	Absorbance	Concentration (%w/v)
0	A	0.272	0.000775
	B	0.485	0.001274
10	A	0.421	0.001124
	B	0.166	0.000527
20	A	0.442	0.001173
	B	0.565	0.001461

4.6 Determination of solubility of Vitamin E in solvents of varying polarity

The calibration curve that was constructed in 4.4 was used to calculate the concentration of the following solutions. Example of calculation is shown in the appendix.

Table 12 Concentrations of Vitamin E in different solvents

Concentration of water (%v/v)	Replicate	Absorbance	Concentration (%w/v)
0	A	0.450	0.001193
	B	0.151	0.000493
10	A	0.232	0.000683

	B	0.146	0.000481
20	A	0.099	0.000371
	B	0.145	0.000479

Chapter 5

Discussion

The average lambda max of vitamin C was found to be 266.45 nm. Kleszczewska and Misiuk found lambda max of ascorbic acid in aqueous solution at 266 nm (Kleszczewska et al., n.d.). The results in our study agree with that. Kleszczewska and Misiuk determined A_1^1 to be 596 at 266 nm. The A_1^1 was found to be 713 in our study. This could be due to error in weighing the small amount of ascorbic acid powder or error in preparing the solutions. In addition, ascorbic acid is ionizable. It would be better to use buffers as solvent rather than distilled water.

The relative standard deviation in the lambda max obtained for different solutions was 0.0022%. The relative standard deviation obtained in the absorbance at lambda max for different solutions was 0.017%. There was not much variation due to changes in the composition of the solvent. This could be due to similar hydrogen bonding capacity of water and acetonitrile (Water | H2O - PubChem, n.d.) (Acetonitrile | CH3CN - PubChem, n.d.). However, there is a trend in increasing lambda max with increasing concentration of acetonitrile in the solvent. There is also a trend in decreasing absorbance with increasing concentration of acetonitrile in the solvent. More conclusive result can be obtained by further increasing proportion of acetonitrile in the solvents and by measuring multiple solutions of the same composition.

Murakami.et al found lambda max of vitamin E at 284.8 nm (Murakami et al., 2003). In this study, the average lambda max was at 285.15nm. Murakami.et al found A_1^1 42.9 at 284nm. A_1^1 was found to be 40.98 in this study. This could be because the solvent used in our study was acetonitrile and they used ethanol as the solvent.

The relative standard deviation in the lambda max obtained for different solutions was 0.11%. The relative standard deviation obtained in the absorbance at lambda max for different solutions was 0.13%. There was not much variation due to changes in the polarity of solvent. However, there is a trend in increasing lambda max with increasing concentration of water in the solvent. There is also a trend in decreasing absorbance with increasing concentration of water in the solvent. More conclusive result can be obtained by further increasing proportion of acetonitrile in the solvents and by measuring multiple solutions of the same composition.

When vitamin E was dissolved in 40% v/v water/acetonitrile solution, the solution became turbid. It is not possible to quantify vitamin E in acetonitrile solution containing more the 20% v/v water using UV-Vis spectroscopy because the solvent becomes too polar to sufficiently dissolve the vitamin E.

Figure 9: Vitamin E containing 40%v/v distilled water.

According to the USP 40 the R^2 value should not be less than 0.995 (United States Pharmacopeial Convention, 2017). In the calibration curve for vitamin C, R^2 was found to be 0.9859 and in the calibration curve for vitamin E, R^2 was found to be 0.9986. The method developed vitamin E compiles with the linearity specification mentioned in the USP. The method for vitamin C does not comply.

According to the USP 40, the relative standard deviation should not be more than 1% for assessing repeatability. In this study, RSD for the vitamin C replicates was 4.7% and for the vitamin E replicates was 1.8%. Neither of these two methods complies with repeatability specification mentioned in the USP.

Reason for this could be attributed to error in pipetting the small volumes. This can be overcome by pipetting larger volumes. Larger weights should be taken to prepare solutions. Vitamin E was very viscous, and this could have incurred error in weighing vitamin E.

In the solubility experiments, the average concentration of the duplicates was calculated and plotted versus solvent compositions. The following trends were observed.

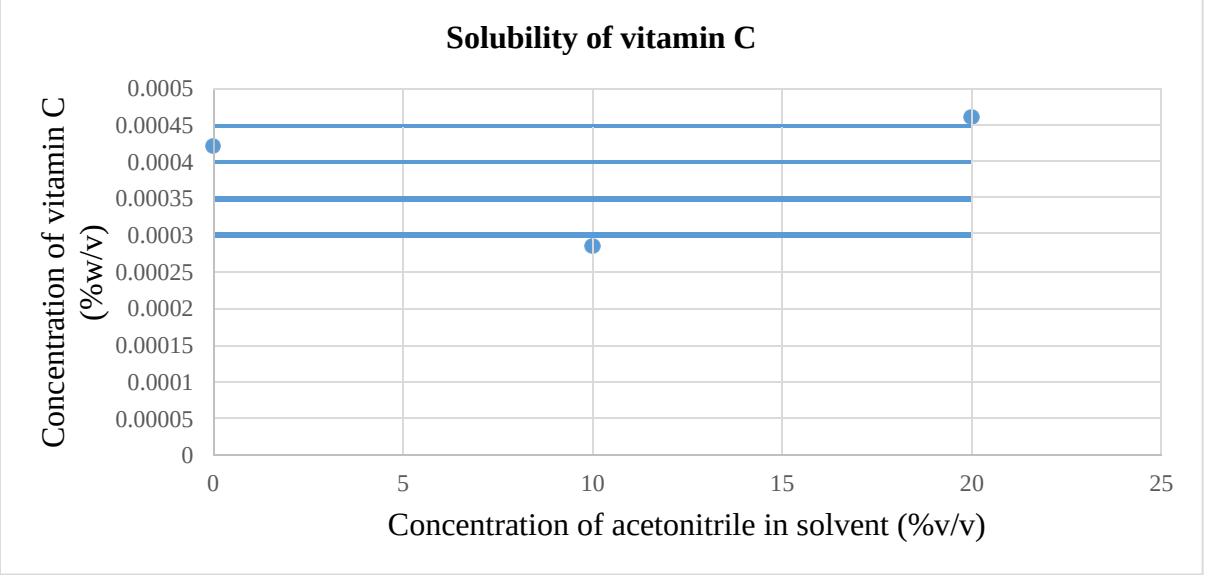


Figure 10 : Change of solubility of vitamin C

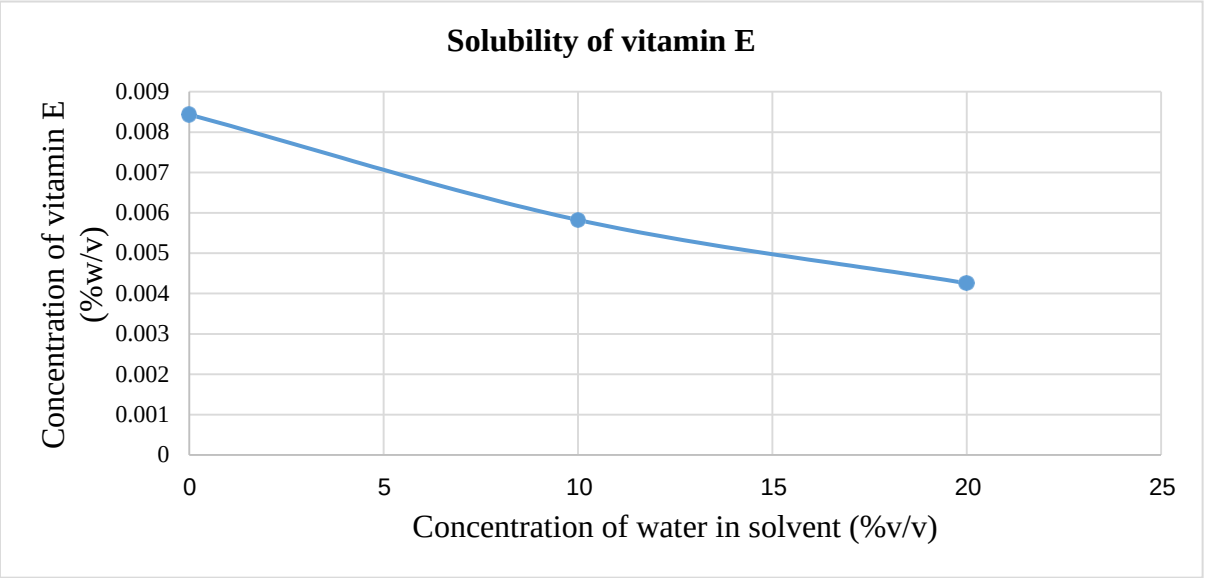


Figure 11: Change of solubility of vitamin E

Omar and Urich observed that the solubility of vitamin C decreases when the polarity of solvent decreases (Omar & Urich, 2006). In our experiment, no trend was observed. Some errors were identified in performing the experiment. Two hours of shaking time was not long enough to obtain equilibrium. When pipetting the supernatant, precipitate might have been pipetted as well. This could give false and high solubility value. Addition of solute was stopped when precipitate was seen after vortexing. This could be before equilibrium was attained.

The solubility of vitamin E decreased with increasing polarity of the solvent. Authors Dubbs and Gupta observed an increase in solubility of vitamin E when the proportion of ethanol in an aqueous solution was increased (Dubbs & Gupta, 1998). Therefore, the results of this study are in agreement with those studies. However, there was larger variation in the concentrations of the replicates. So, the results are not conclusive.

Chapter 6

Conclusion

This project included six experiments. Methods were developed and validated. These methods were then used. During this experiment, some performance error was occurred. These problems can be overcome by doing this experiment again with proper care but due to time constriction this cannot be done twice. The results of this project can be used to design more extensive experiments for the future.

Chapter 7

Future direction

- This project might help to compare data.
- Also this project can be extending by working with different solvents.
- Results of this project can aid in extracting vitamins from multivitamin preparations containing both fat soluble and water-soluble vitamins.

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Appendix

Appendix to 3.1

Weight of vitamin C measured to make solution:

	Weight (g)
Paper	0.313
Paper with sample	0.416
Paper without sample	0.305
Sample	0.111

Appendix to 3.2

Weight of vitamin E measured to make solution:

	Weight (g)
Flask	13.664
Flask with sample	13.768
Sample	0.104

Appendix to 4.1

The absorbances at each peak are listed below.

0% v/v acetonitrile in solvent

No.	Wavelength (nm)	Absorbance	Comment

1.	339.80	-0.011	
2.	266.20	0.143	Taken as lamda max
3.	233.80	0.034	

5% v/v acetonitrile in solvent

No.	Wavelength (nm)	Absorbance	Comments
1.	339.80	-0.013	
2.	265.80	1.589	Taken as lamda max
3.	338.60	-0.014	
4.	215.00	0.105	

10% v/v acetonitrile in solvent

No.	Wavelength (nm)	Absorbance	Comments
1.	339.60	-0.013	
2.	266.60	1.615	Taken as lamda max
3.	338.80	-0.015	
4.	216.00	0.121	

20% v/v acetonitrile in solvent

No.	Wavelength (nm)	Absorbance	Comments
1.	339.80	-0.011	
2.	266.20	1.585	Taken as lamda max
3.	216.20	0.180	

40% v/v acetonitrile in solvent.

No.	Wavelength (nm)	Absorbance	Comments
1.	339.60	0.001	
2.	267.20	1.548	Taken as lambda max
3.	228.20	0.470	
4.	338.80	-0.001	
5.	233.40	0.459	
6.	214.80	0.380	

The lambda max selected for the future experiment was calculated from the average.

%v/v acetonitrile in solvent	Wavelength (nm)	Absorbance
5	265.80	1.589
10	266.60	1.615
20	266.20	1.585
40	267.20	1.548
	Average = 266.45	Average = 1.58

Appendix to 4.2

The absorbance at each peak are listed below.

0% v/v water in solvent

No.	Wavelength (nm)	Absorbance	Comments

1.	340.00	0.059	
2.	285.40	0.780	Taken as lambda max.
3.	279.00	0.690	
4.	223.60	2.704	
5.	215.00	3.000	
6.	338.40	0.057	
7.	280.20	0.687	
8	253.20	0.154	
9	217.60	2.562	
10.	201.20	1.344	

5% v/v water in solvent

No.	Wavelength (nm)	Absorbance	Comments
1.	340.40	0.043	
2	285.40	0.739	Taken as lambda max.
3	278.60	0.654	
4	224.20	2.635	
5	218.60	2.596	
6	214.40	2.918	
7	338.80	0.041	
8	280.20	0.650	
9	253.00	0.135	
10	219.40	2.561	
11	217.60	2.528	

12	202.20	1.134	

10% v/v water in solvent

No.	Wavelength (nm)	Absorbance	Comments
1	340.40	0.028	
2	285.00	0.922	Taken as lambda max.
3	278.80	0.814	
4	226.00	2.793	
5	215.20	3.171	
6	338.40	0.026	
7	280.00	0.810	
8	253.00	0.154	
9	217.60	2.605	
10	205.60	1.535	

20% v/v water in solvent

No.	Wavelength (nm)	Absorbance	Comments
1	284.80	0.969	Taken as lambda max
2	278.60	0.867	
3	228.20	2.832	
4	224.40	2.822	
5	214.80	3.271	
6	202.20	3.465	

7	280.00	0.863	
8	253.80	0.234	
9	225.20	2.775	
10	218.00	2.628	
11	203.60	1.589	

40% v/v water in solvent.

No.	Wavelength (nm)	Absorbance	Comments
1	285.80	2.235	Taken as lambda max
2	230.40	4.000	
3	222.40	3.999	
4	260.20	1.737	
5	225.40	3.649	

The lamda max selected for the future experiment was calculated from the average.

%v/v water in solvent	Wavelength (nm)	Absorbance
0	285.40	0.780
5	285.40	0.739
10	285.00	0.922
20	284.80	0.969
	Average = 285.4	Average = 0.85

Appendix to 4.3

Calculation for initial concentration:

Weight of vitamin C taken = 0.111g

Volume of solution = 10ml

If 10ml contains 0.111g

100 mL will contain 1.11g

Therefore, initial concentration is 1.11 %w/v

Calculation of final concentration:

Solution	Total dilution	Final concentration
1	10*10*(10/1)= 1000	1.11/1000= 0.00111
2	10*10*(10/1.3)=769.2307	1.11/769.2307=0.00144
3	10*10*(10/1.4)=714.2857	1.11/714.2857=0.00155
4	10*10*(10/1.5)=666.6667	1.11/666.6667=0.00166
5	10*10*(10/1.7)=588.2353	1.11/588.2353=0.00189
6	10*10*(10/2)=500	1.11/500=0.00222

Example of calculation of concentration using calibration curve:

Equation of line, y = 469.29x + 0.287

Upon rearranging, we get x = (y-0.287)/469.29

Where, x = concentration and y = absorbance

Therefore, if absorbance = 1.093, concentration = 0.001718%w/v

Appendix to 4.4

Calculation of concentration:

Weight of vitamin E taken = 0.104g

Volume = 10ml

10ml contains 0.104g

100 contains 1.04g

Initial concentration 1.04 %w/v

Solution	Total dilution	Final concentration
0.5	10/0.5*4=80	1.04/80=0.013
0.6	10/0.6*4=66.66667	1.04/66.66667=0.0156
1	10/1*4=40	1.04/40=0.026
1.1	10/1.1*4=36.36364	1.04/36.36364=0.0286
1.2	10/1.2*4=33.33333	1.04/33.33333=0.0312

Example calculation of concentration using calibration curve:

Equation of line: y = 427.1x – 0.0596

Upon rearranging, we get: x= (0.0596+y)/ 427.1

Where, x = concentration and y = absorbance

Therefore, if absorbance = 1.291, concentration = 0.003162%w/v

Appendix to 5

Calculation of average concentration of the replicates for vitamin C

Concentration of acetonitrile (%v/v)	Replicate	Absorbance	Concentration (%w/v)	Average concentration (%w/v)	Standard deviation
0	A	0.272	-3.19632E-05	0.0004219	-
	B	0.485	0.000421914		
10	A	0.421	0.000285538	0.0002855	-
	B	0.166	-0.000257836		
20	A	0.442	0.000330286	0.0004610	0.000185
	B	0.565	0.000592384		

Calculation of average concentration of the replicates for vitamin E

Concentration of water (%v/v)	Replicate	Absorbance	Concentration (%w/v)	Average concentration (%w/v)	Standard deviation
0	A	0.450	0.001193	0.000843	0.000494975
	B	0.151	0.000493		
10	A	0.232	0.000683	0.000582	0.000142836
	B	0.146	0.000481		
20	A	0.099	0.000371	0.000425	7.63675E-05
	B	0.145	0.000479		