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Research Article

Process for Preparation of Vitamin C and Method for Determination of Vitamin C in Tablets

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Received: June 05, 2018; **Published:** June 15, 2018

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Abstract

Procedure for calcium ascorbate (VITAMIN-C) includes such technological steps as reaction of ascorbic acid on calcium carbonate in water. In recent years, the determination of vitamin C has become an important subject in the field of biochemistry and commercial foods. This is because vitamin C plays an important role in maintaining human health. Due to the importance of vitamin C in human beings, the quantitative analysis of vitamin C has gained a significant increase in several areas of analytical chemistry such as pharmaceutical and food applications. There are numerous methods for the determination of vitamin C in a variety of natural samples, biological fluids and pharmaceutical formulations. The prepartaion method and methods for the determination of vitamin C are spectrophotometric methods and non-spectrophotometric methods (Arya and Mahajan, 1997). For non-spectrophotometric methods are such as high-performance liquid chromatography (HPLC), titration, enzymatic method and fluorometry (Arya, Mahajan and Jain, 2000). Direct spectrophotometry also has been applied to determine the vitamin C content in soft drinks, fruit juices, and cordials after correction for background absorption in the UV region.

Abbreviations: FIA: Flow Injection Analysis; HPLC: High Performance Liquid Chromatography HMF: Hydroxymethylfurfural; AIDS: Acquired Immuno Deficiency Syndrome; FDA: Food and Drug Administration

Introduction

Ascorbic acid (vitamin C) is a water-soluble vitamin. It occurs as a white or slightly yellow crystal or powder with a slight acidic taste. It is an antiscorbutic product. On exposure to light, it gradually darkens. In the dry state, it is reasonably stable in air, but in solution it rapidly oxidizes. Ascorbic acid (vitamin c) is freely soluble in water; sparingly soluble in alcohol; insoluble in chloroform, in ether, and in benzene. The chemical name of ascorbic acid (vitamin c) is L-ascorbic acid (vitamin c). The empirical formula is $C_6H_8O_6$, and the molecular weight is 176.13. The synthesis of ascorbic acid was achieved by Reichstein in 1933, followed by industrial production of ascorbic acid two years later by Roche. Today, vitamin C identical to that occurring in nature is produced on a very large industrial scale. The ultimate raw material for the production of vitamin C (ascorbic acid) is corn or wheat. This is converted via starch to glucose by specialist companies, and then to sorbitol. We produce the pure final products from sorbitol in a series of biotechnical, chemical processing and purification steps.

Vitamin C = Ascorbic Acid

Empirical formula: $C_6H_8O_6$

Molecular weight: 176.1

Melting point: about 190 °C (with decomposition)

Appearance: white to slightly yellowish crystalline powder, practically odorless, with a strong acidic taste.

The water-soluble vitamin C is probably the most well-known vitamin. Even before its discovery in 1932, physicians recognized that there must be a compound in citrus fruits preventing scurvy, a disease that killed as many as two million sailors between 1500 and 1800. Later researchers. Discovered that man, other primates and the guinea pig depend on external sources to cover their vitamin C requirements. Most other animals are able to synthesize vitamin C from glucose and galactose in their bodies. Nowadays, health has become the most important property of human's life. Commonly, diets with high contents of fruits are protective against several human diseases such as cardiovascular diseases and even cancer. Therefore, people are putting more and more attention on

antioxidant substances such as vitamin C which is also known as ascorbic acid or more specifically L-ascorbic acid.

Vitamin C is probably one of the most highly well known. Furthermore, people have become more aware to the importance of vitamin C. Hence, this causes the global market flooded with vitamin C fortified foods (Arya, Mahajan and Jain, 2000). The term of vitamin C is used as generic term for all compounds exhibiting qualitatively the biological activity of ascorbic acid. The molecular structure of vitamin C is $C_6H_8O_6$ and the molecular weight is 176.1 (Ball, 2006). Vitamin C is highly polar and readily soluble in aqueous solution and insoluble in less nonpolar solvents (Fennema, 1996). It is an acidic compound due to the facile ionization of hydroxyl group on carbon 3 (pK1 = 4.17) while the hydroxyl group on carbon 2 is much more resistant to ionization (pK2 = 11.79).

The structure of L-ascorbic acid is shown in Figure 1 (Ball, 2006). Ball (2006) also stated that ascorbic acid is easily and reversibly oxidized to dehydroascorbic acid, forming the ascorbyl radical anion which is also known as semidehydoascorbate as an intermediate as shown in Figure 2. Dehydoascorbic acid possesses full vitamin C activity because it is readily reduced to ascorbic acid in the animal body. However, dehydoascorbic acid is not an acid in the chemical sense, as it does not have the dissociable protons that ascorbic acid has at carbon 2 and carbon 3 positions.

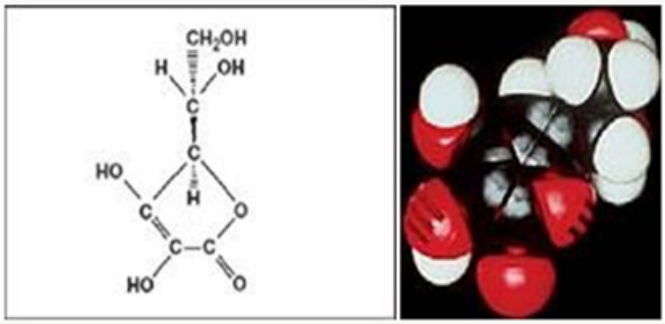

Figure 1: structure of L-ascorbic acid.

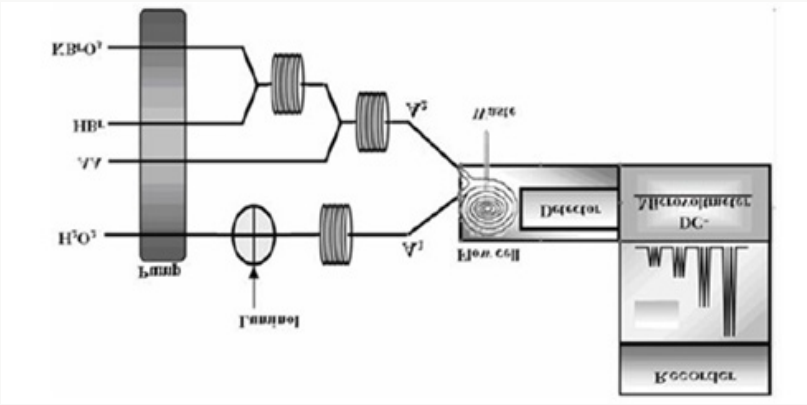

Figure 2: Schematic diagram of the rFIA-CL manifold used in this study for the determination of ascorbic acid.

One of the most important properties of vitamin C is that it is an antioxidant. Nevertheless, it has a wide range of antioxidant properties outside the body and can quench most biologically active radicals. It scavenges superoxide, nitroxide, hydroxide, hydrogen peroxide and will reduce vitamin E (Hickey and Roberts, 2004). It is also found to be a strong antioxidant as it helps to neutralize harmful free radicals (Izuagie and Izuagie, 2007). Vitamin C is an almost odorless white or pale yellow crystalline powder with a pleasant sharp taste and melting point of about 190°C. It is not a carboxylic acid but a lactone and ease of oxidation to the presence of an enediol grouping (Izuagie and Izuagie, 2007). Vitamin C is highly susceptible to oxidation, especially when catalyzed by metal ions such as copper (II) ion and iron(III) ion. The functions and activities of vitamin C are based on its properties as a reversible biological reductant (Hickey and Roberts, 2004) (Figure 1).

Vitamin C participates for the growth and repair of tissues in all parts of the body (Kleszczewski and Kleszczewska 2002). Vitamin C is a natural antioxidant that mostly found in fruits and vegetables. The main sources of vitamin C are citrus fruits, strawberries, peppers, tomatoes, cabbage, and spinach. Vitamin C plays crucial roles in electron transport, hydroxylation reactions and oxidative catabolism of aromatic compounds in animal metabolism (Gazdik, 2008). Vitamin C can help to prevent and treat common cold, mental illness, infertility, scurvy, cancer and Acquired Immune Deficiency Syndrome (AIDS) (Yusuf and Gurel, 2005). It is reported to lower cancer risk and also said to have important interactions with other vitamins. For example, excessive intake of vitamin A is less toxic to the body when vitamin C is readily available (Izuagie and Izuagie, 2007). Due to the great importance of vitamin C in human beings, the quantitative analysis of vitamin C has gained

increased significance in several areas of analytical chemistry such as pharmaceutical and food applications (Yusuf and Gurel, 2005).

Vitamin C is also used as an index of the nutrient quality for fruit and vegetable products. This is because it is much more sensitive to various modes of degradation in food processing and subsequent storage (Ozkan, Kirca and Cemero, 2004). It is well known that vitamin C is easily oxidized to dehydroascorbic acid in alkaline solutions, while it is relatively stable in acidic solution. Vitamin C of fruit juices is readily oxidized and lost during staying of the juices (Kabasakalis, Siopidou and Moshatou, 2000). In the food industry, vitamin C is used as food additive (Mai and Mohammed, 2004). It preserves and protects food from any colour changes and act as an important component of our nutrition as well. Vitamin C helps to prevent the degradation of soft drinks and juice which helps to retain their flavors. Hence, it increases the quality of food and nutritional value as well (Burdurlu, Koca and Karadeniz, 2005).

Degradation of vitamin C undergoes both anaerobic and aerobic pathways. Qxidation of vitamin C in aerobic pathway occurs mainly during the processing of food whereas anaerobic degradation of vitamin C mainly during storage. Hydroxymethylfurfural (HMF) is one of the decomposition products of vitamin C and acts as precursor of brown pigments (Burdurlu, Koca and Karadeniz, 2005). Vitamin C degradation in packaged fruit juices depends mainly on storage temperature, pH, dissolved oxygen level, residual hydrogen peroxide, H_2O_2 left after the sterilization of packaging material and trace metal ions (Ozkan, Kirca and Cameroglu, 2004). Consequently, studies on vitamin C content in foods are important in relation to the control of nutritional labels, the update of food databases and the establishment of dietary reference intakes. Orange juice is probably the most globally accepted fruit juice and it is recognized worldwide as a good source of ascorbic acid (Sharma, Singh and Saxena, 2006).

In addition, there are many analytical methods used to determine the concentration of vitamin C in the pharmaceutical samples which are colorimetric method, titration, enzymatic method, Flow Injection Analysis (FIA) and High Performance Liquid Chromatography (HPLC) (Arya and Mahajan, 1997). Reflectometer is an instrument that can used to analyze many different types of test which include ascorbic acid test that is concerned in this project. It provides a simple and rapid determination of vitamin C content in many pharmaceuticals product.

Procedure for Calcium Ascorbate

Process

a) In 500.0ml RBF fitted with thermometer pocket and stirrer.

b) Take 200.0ml DM water at 25-30 °C.

c) Charge 30.0gm $CaCO_3$ at 25-30 °C.

d) Charge 0.5ml TGA (Thioglycolic acid)

e) Maintain and stir for 1.0 hr at 25-30 °C.

f) Filter the slurry at 25-30 °C.

g) Suck dry and wash with 100.0ml water.

h) Suck dry and unload wet cake.

Wt. of wet cake 42.0gm

Process

a) In 1000.0ml RBF fitted with thermometer pocket and stirrer.

b) Charge 150.0ml water at 25-30°C.

c) Charge 100.0g Ascorbic acid at 25-30°C.

d) Charge 0.2gm EDTA and 0.2ml TGA at 25-30 °C.

e) Maintain and stir reaction mass for 15.0min at 25-30 °C.

f) Charge above wet cake in 30-45min. at 25-30 °C.

g) Maintain and stir reaction mass for 1.0hr. at 25-30 °C. Solution should be hazy.

h) Clarify the reaction mass at 25-30 °C.

i) Collect filtrate

j) In another 1.0lit. RBF fitted with thermometer pocket and stirrer.

k) Take 500.0ml 95.0% methanol (475.0ml methanol and 25.0ml water).

l) Start addition of above filtrate in 30-45min. at 25-30 °C.

m) Maintain and stir reaction mass for 30.0min. at 25-30 °C.

n) Cool reaction mass to 10°C in 30.0 min.

o) Maintain and stir reaction mass for 1.0hr. at 10-15°C.

p) Filter the reaction mass at 10-15°C.

q) Suck dry and wash with 50.0ml methanol at 25-30°C.

r) Suck dry and unload wet cake.

Wt. of wet cake 100-110.0gm Dry at 40°C under vacuum LOD NMT 0.1% Wt. of dry material 90.0gm (Tables 1 & 2).

Table 1.

Volumetric flask 250.00 ml (± 0.01ml)
Beaker 500.00ml (±0.01ml), 500.00ml (±0.01ml), 100ml (±0.01ml)
Beaker 500.00ml (±0.01 ml), 500.00ml (±0.01ml), 100 ml (±0.01ml)
Graduated cylinder 50.00ml (±0.01ml
Erlenmeyer flask 250.00ml (± 0.01ml), Erlenmeyer flask 250.00ml
Potassium iodide (KI) 15.00g (± 0.001g)

Iodine powder 5g
Balance

Table 2.

Starch powder 0.25g
Distilled water
Heater Glass rod
Vitamin C tablet (ACI Limited - Nutrivit® C)
Variables and constants
Constants Temperature: 25-260C (measured with electronic thermometer) Sun light (direct sunlight damages the structure of vitamin C) Independent Variable Unknown amount of vitamin C in solution. Dependent Variable The amount of iodate solution used to reach the end point of reaction between vitamin C and iodate.

Experimental Procedures

Ascorbic acid is determined by using an oxidation-reduction reaction. The solubility of iodine is increased with iodide and tri-iodide is occurred:

I2 (aq) + I↔ I3 - I3 -

Then oxidizes vitamin C to dehydroascorbic acid: $C_6H_8O_6$ + I3 + H_2O → $C_6H_6O_6$ + 3I- + 2H+ Vitamin C dehydroascorbic acid The endpoint is production of a blue-black color which occurs as a result of the reaction of iodine with starch suspension. When ascorbic acid is present, I3 is converted to iodide and no color change is observed. However, when all ascorbic acid was utilized, expected blue-black color occurs due to the reaction between starch and excess tri-iodide. This titration procedure is widely accepted and is appropriate for testing the amount of vitamin C in the tablets, liquids and fruits and vegetables [1].

Preparation of Iodine Solution

For preparation of 0.1 M iodine solution, 10g of KI was taken in a 250ml Volumetric flask and 35 ml of distilled water was added followed by heating the solution; the mixture was cooled to room temperature and 3.15g of solid Iodine powder was dissolved. Similarly, to prepare 0.005M of iodine solution 2g of KI was taken in a 500ml beaker and dissolving in 100 ml of distilled water and 1.3 g of iodine powder was stirred with small quantity of water and qs (quantum satis) to 1 litre [2].

Preparation of Starch Solution

Addition of 0.25g of starch powder in 50ml warm distilled water, As the starch is insoluble in cold water and needs to be boiled to stay in solution [3].

Preparation of Vitamin C Standard Solution

25mg Ascorbic acid was taken in a 100.00ml beaker and dissolved in 100 ml distilled water [4].

Preparation of Vitamin C Sample Solution

From the strip of Vitamin C random two tablets were weighed and smashed to form powder and average value was calculated.

334mg of the powder tablet was taken in a 100.00ml beaker and dissolved in 100ml distilled water [5] (Table 3). Standardization of the iodine solution with the vitamin C standard solution and sample solution. The measured volume of 20ml of both standard and sample was taken from each solution and equilibrated with 150ml distilled water separately into distinct two Erlenmeyer flask 250.00ml and titrant containing iodine solution was run against analyte containing either sample or standard; 5-6 drops of prepared starch solution were added to the analyte and titration was started. The burette level for each analyte for distinctive experiment was noted as mentioned below: For standard solution the volume of iodine solution required for complete reaction = 45ml Equally, for Sample solution the volume of iodine solution required = 49ml The endpoint was noted when analyte appears blue in color [4].

Table 3.

Weight of two tablets = 1.67g
Average = 1.67/2 = 835mg
250 mg Ascorbic acid tablet has equivalent weight of = 835mg
100mg Ascorbic acid tablet has equivalent weight of = 167*2mg = 334mg

Calculation

For sample solution in the beginning of the experiment 20ml of sample was taken from 100ml of prepared solution containing 100mg of Ascorbic acid. As 49ml of iodine is required for the color change containing 20ml ascorbic acid solution, the dilution was done 5 times to that of the solution. Hence, the final volume of the iodine solution = 49× 5 = 245 ml

Mole iodine = Mass Ascorbic acid × 1 mole 176.12g of ascorbic acid × 1000ml volume of iodine M iodine = 0.1g × 1/176.12 × 1000 245ml = 0.00231 mol

For standard solution Mass Ascorbic acid = Mole iodine × Volume of iodine × 176.12 = 0.00231 × 45 × 176.12 = 91.54 mg Initially, the amount of Ascorbic acid was taken for 100mg and therefore for total amount of ascorbic acid i.e. 250 mg the ratio stands out to be 2.5 (250/100).

Result

Therefore, a 250mg tablet of ascorbic acid from the ACI LIMITED (Nutrivit® C) contain = 2.5 × 91.54 = 228.85 mg Amount of Ascorbic acid in ACI LIMITED (Nutrivit® C) is 8.46% less than the claimed value. %

Flow Injection Analysis (FIA)

In FIA, there is no air segmentation and it is not necessary for a state of chemical equilibrium to be reached. The sample is introduced into a carrier stream as a discrete plug. The presence of a sample-carrier interface allows diffusion-controlled dispersion of the sample as it is swept through narrow-bore tubing to create a concentration gradient. The flow-through detector monitors the change in concentration of the reaction product, which is displayed

as a well-defined peak (Ball, 2006). Flow-Injection analysis permits a simple, rapid and sensitive method for the determination of vitamin C where its systems allow faster sampling rates and consumed fewer reagents compared with segmented-flow analysis (Kleszczewki and Kleszczewska, 2002).

Memon, Dahot and Ansari had proposed a method by using mono 1, 10-phenanthroline-iron(III) complex as oxidant. This experiment was based on its reducing reaction on mono(1-10-Phenanthroline)-iron(III) to tris(1,10-Phenanthroline)-iron(II) (ferroin) and the absorbance of ferroin was monitored at 510nm through spectrophotometer equipped with a flow through cell [6] (Figure 2). In this analysis single channel manifold is used as shown in Figure 2. The reagent stream is pumped at the flow rate 1.1mL/min via a peristaltic pump equipped with PVC pump tubing. The vitamin C sample is introduced into the reagent stream via a rotary teflon valve. A calibration curve for vitamin C in the range 0-50ppm was plotted from the results obtained by Memon, Memon, Dahot and Ansari which are shown in Figure 3. They also studied about the effect of reaction coil and reagent concentration. From the graph (Figure 3), the maximum intensity was observed at 50cm reaction coil (Figure 4).

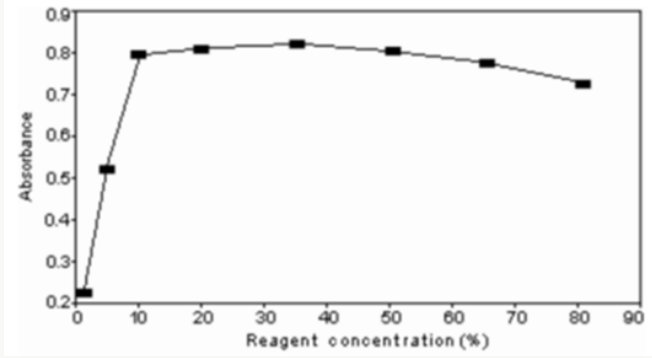

Figure 3

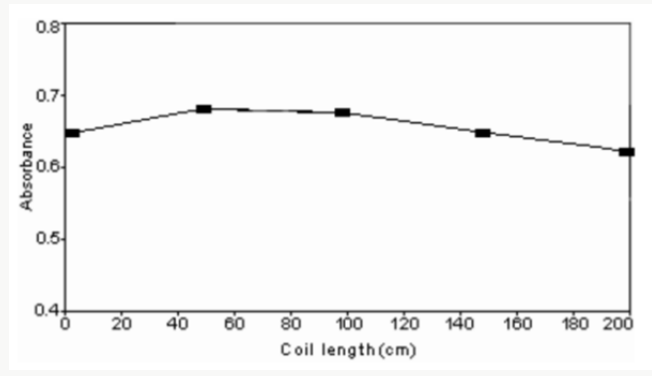

Figure 4

While the results of the effect of reagent concentration obtained is shown in Figure 5 indicating that the maximum signal could be obtained at 35% reagent (Memon, Memon, Dahot and Ansari, 2000). This method can be improved within certain limits by increasing the volume of the injected sample in flow injection analysis. The sensitivity is increased two fold with the increase of sample volume. As conclusion, since the time required for sample preparation is short and reagent consumption is low, hence the method is highly economical and is suitable to use on routine basis for determination of ascorbic acid in pharmaceutical preparations [7].

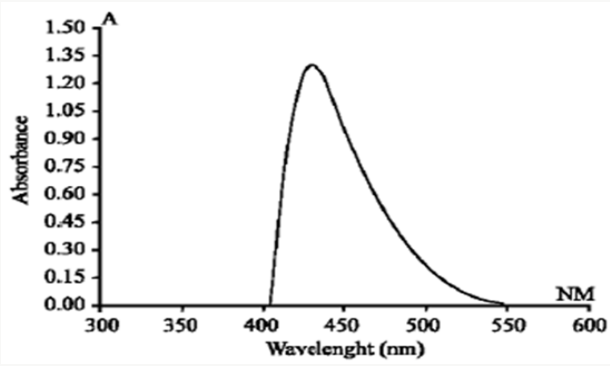

Figure 5

Ultraviolet (UV) Spectrophotometry

Direct ultraviolet spectrophotometry is a fast, simple and reliable method for the determination of vitamin C. This method can be done through alkaline treatment and the maximum absorption of vitamin C falls at 243nm at pH2 (Yanshan, 1997). The absorption of UV light by the sample matrix was the major problem in this method. Therefore, alkaline treatment method was found to be used as background correction in blank. This is because more than 95% of vitamin C will be destroyed in 10 minutes after alkaline treatment which is in the range of pH 12 to 13 (Salkic and Kubicek, 2008). UV spectrophotometry method was found to be applicable for most fruits, fruit juices and soft drinks except those that are unstable to alkaline treatment, and were deeply colored, or contained high concentration of caffeine, saccharin, caramel and tannic acid (Yanshan, 1997).

To determine the total content of vitamin C in food samples, a well-established method was investigated by Khan, Rahman, Islam and Begum, 2006 by using the 2,4-dinitrophenyl hydrazine methods (DNPH). This is a simplified method for the simultaneous determination of total vitamin C employed coupling reaction of 2,4-dinitrophenyl hydrazine dye with vitamin C and followed by spectrophotometric determination. The spectrophotometric method involves the oxidation of ascorbic acid to dehydroascorbic acid by the action of bromine solution in the presence of acetic acid. Reaction between dehydoascorbic acid and 2,4-dinitrophenyl hydrazine at 37 °C temperature for three hours will form an osazone. The solution is treated with 85% H2SO4 to produce a red color complex.

The absorbance of all standards was measured at 521nm by using a UV-spectrophotometer. The results obtained were taken to contruct a calibration curve (Khan et al, 2006) (Figure 5). The calibration curve was constructed by plotting the concentration versus the corresponding absorbance as shown by Figure 6. The molar absorptivity? Can be obtained using Beer-Lambert plots.

The reliabilty of this method was justified by the calculations of the % of standard deviation and it was found to be varied within the range from 0.20 to 2.45%. The reliability of this method was also confirmed from the consideration of the following expected interferences [8].

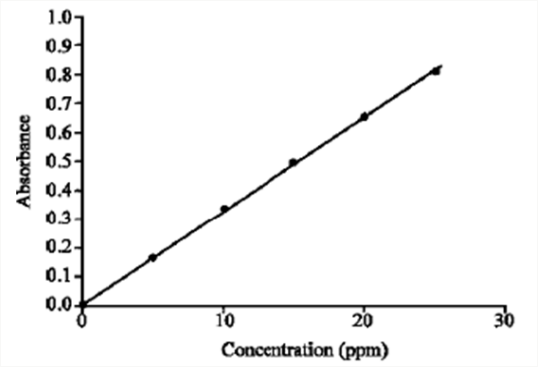

Figure 6

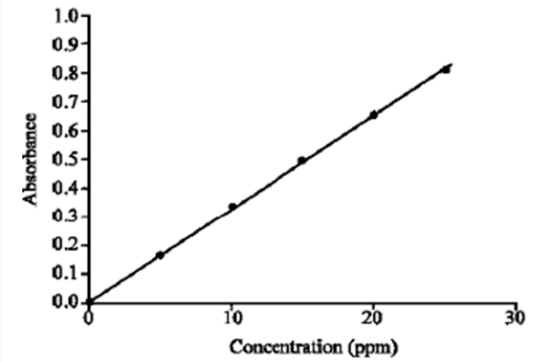

Figure 6.1

There are a few interferences that might affect the results. First, the interference was due to the diketogulonic acid. At higher pH, destructive oxidation hydrolysis might occur (Figure 6). This result in the opening of the lactone ring of the ascorbic acid and loose the vitamin activity. These processes are naturally occurred in fruits and some amounts of diketogulonic acid are presence in the fruits. Besides that, diketogulonic acid has keto group that might form osazone when react with DNPH. Hence, there is a chance of error in this method which may give false results (Khan et al, 2006) [9]. Another interference was due to the extracted glucose which contains similar structure like vitamin C. Therefore, some of the glucose may be extracted in the meta-phosphoric acid during the extraction of ascorbic acid from sample. Glucose may also cause the formation of colored complex with DNPH and gives the false result in the determination of vitamin C. This was proven in Figure 6.1 where there is no absorption peak around the interested peak at 52nm (Khan et al, 2006). As conclusion, the method is simple and excellent for the determination of total vitamin C in fruits and vegetables (Figure 6.1).

Fluorometric Method

Fluorometric analysis has been used for ascorbic acid assay in pharmaceutical preparations, beverages, special dietary foods and even for human serum (Arya, Mahajan and Jain, 2000). This method had been reported to have successful application to a wide range of foodstuffs, including liver, milk, fresh and canned fruit, raw and cooked vegetables, and potato powder (Ball, 2006). Previously, fluorometric determinations of vitamin C have been developed based on condensation reactions of vitamin C with o-phenylenediamine and on the oxidation with mercury (II) of vitamin C to form quinoxaline derivative. The reaction products of these methods exhibit fluorescensce (Yusuf and Gurel, 2005) [10].

Figure 7 shows the reaction of the dehydroascorbic acid with 1,2-phenylenediamine dihydrochloride to form the fluorescent quinoxaline derivative 3(1,2dihydroxyethyl) furol [3,4-b] quinoxaline-1-one. The blank can be prepared by complexing the oxidized vitamin with boric acid to prevent the formation of the quinoxaline derivative. It is used to reveal any fluorescence due to interfering substances (Ball, 2006). (Figures 7 & 8). Yusuf and Gurel have described a method by using Methylene Blue (MB) for the determination of vitamin C. This experiment was run by using a spectrofluorimeter to record the spectra and carry out fluorescence measurements [11].

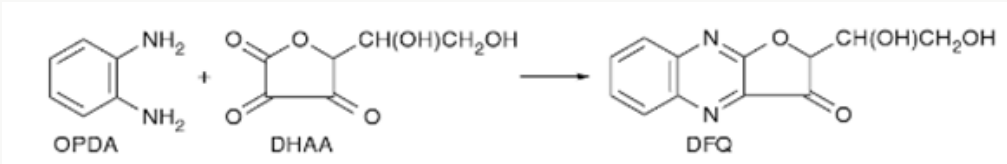

Figure 7

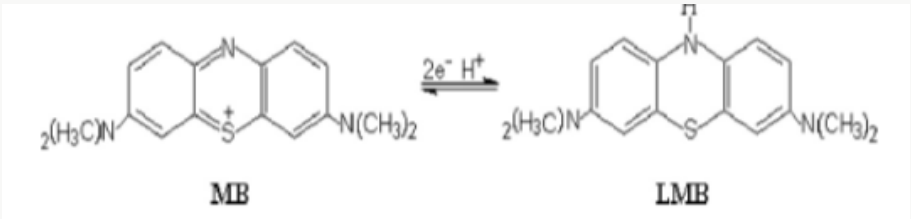

Figure 8

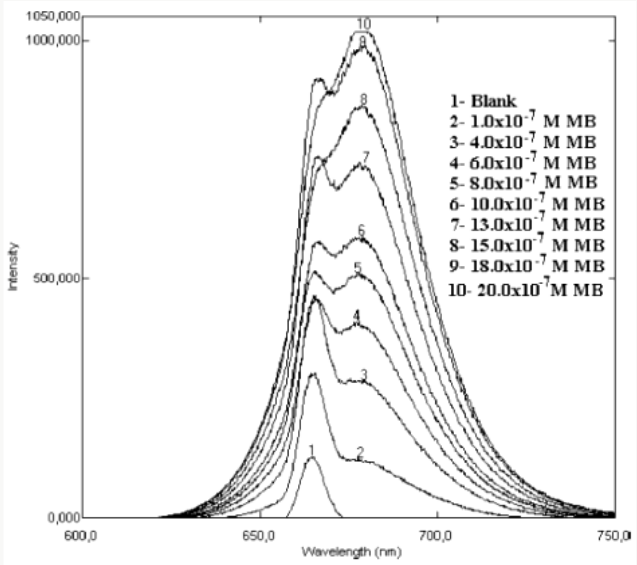

Figure 9

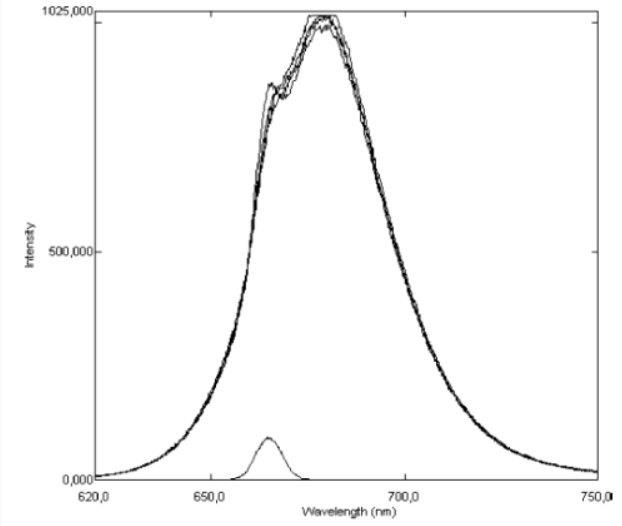

Figure 10

This method was used to determine the amount of vitamin C in the purified materials, specifically vitamin C tablets. MB is a member of thiazine dye group. It is widely used in many different areas. For example, a photo sensitizer is used to produce singlet oxygen in photodynamic therapy for the treatment of cancer [12]. The highly colored oxidized form of MB can be reduced to be colorless leuco form, Leuco-Methylene Blue (LMB) which is shown in Figure 9. LMB is the reduced and colorless form of methylene blue (Yusuf and Gurel, 2005) (Figure 9). According to Yusuf and Gurel, the fluorescence bands of MB were obtained at 664nm for excitation state and 682nm for emission peaks. This was proven by the other researchers who also examined the emission bands at 682nm for MB and 452nm for LMB. In Figure 10, the emission peak of MB at 682nm increased due to the increase of its concentration. A linear relationship between MB concentration and intensity was obtained over the concentration range of mol L-1 MB (y= 49.082x + 94.46,r2=0.9969). The excitation peak of MB at 664nm also linearly increased depending on the increase of its concentration (Yusuf and Gurel, 2005) (Figure 10). The studies of the effect of vitamin C on the fluorescence of MB is made to avoid any errors that might affect the accuracy of the results. In order to examine the effect of vitamin C on the fluorescence of MB at 664 nm, mol L-1 MB solutions, each solution was added with different concentration of vitamin C and were prepared under nitrogen (N2) atmosphere. This was shown in Figure 11 where the spectra were recorded at 664nm (Yusuf and Gurel, 2005). Figure 13 above shows the excitation intensity of mol L-1 without adding vitamin C was about 1000.0 and above. The intensity was decreased by the increase of vitamin C concentration in MB solutions (Yusuf and Gurel, 2005) (Figure 11).

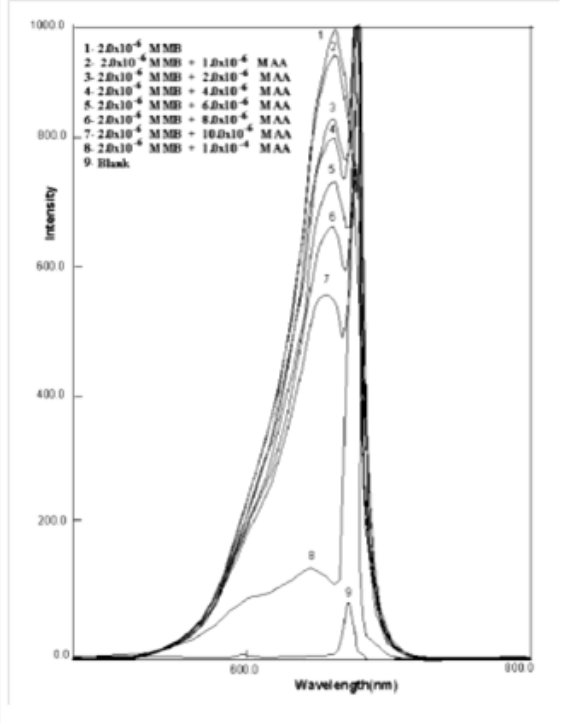

Figure 11

Table 4: Tolerance between Different Substances in Determination of Ascorbic acida).

Additive Type	Maximum Tolerable Concentration ratio
Glucose, Fructose, Alanin, Saccharose, Urea, Acetat, Benzoic acid	100
Citric acid	20

Note: Ascorbic acid concentration 1.0 X 10-6 mol^{-1}

Figure 9 shows the emission spectrum of mol L^{-1} MB as a function of time. Each spectrum was recorded at 1 minute intervals. The results showed that the fluorescence was not changed with time, reflecting that the fluorescence spectrum of MB was highly stable with time (Yusuf and Gurel, 2005) (Figure 12). In the redox reaction between ascorbic acid and MB, the ascorbic acid is oxidized

to dehydroascorbic acid, while MB was reduced to colorless LMB as shown in the following: The calibration curve was made based on the concentration of MB (mol L_{-1})[13]. The results indicate that the fluorescence intensity of the system is a linear function of vitamin C concentration in the range of mol L^{-1} and the regression coefficient is 0.9941 as shown in Figure 10 (Yusuf and Gurel, 2005) (Figure 13) (Table 4).

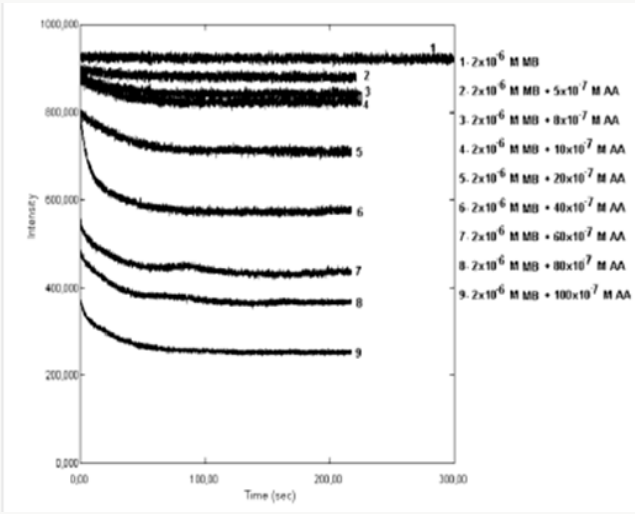

Figure 12

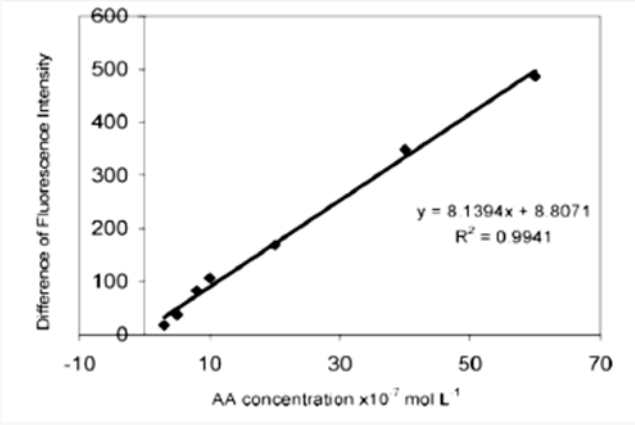

Figure 13

Table 4 below shows the tolerance towards different compounds that might cause interferences in this method. These compounds are usually present in most vitamin C tablets. The experimental results showed that the presence of hundred-fold excess of the all contaminant compounds and twenty-fold excess of citric acid did not significantly influence the determination of vitamin C using this method. Therefore, it can be concluded that there is no major interference caused by these compounds (Yusuf and Gurel, 2005). So it is possible to use this method for direct determination of vitamin C in pharmaceuticals without separating the interfering materials (Table 5).

Table 5 lists the results obtained by the proposed method with triiodide method. It can be clearly seen that the results are in good agreement with the triiodide method (Yusuf and Gurel, 2005). Thus, the proposed method provides a simple and sensitive fluorimetric procedure by using MB for the determination of vitamin C [14,15]. This experiment also shows that MB could be used for fluorimetric determination of vitamin C in vitamin C tablets although it has only slightly fluorescence property compared to LMB. Therefore, as conclusion, it can be explained that the fluorescence intensity of MB was more sensitive to determine vitamin C concentration [16].

Table 5: Determination of Ascorbic acid in Pharmaceutical preparations.

Sample	Amount of Ascorbic acid (mgg^{-1})	
	Proposed method[a])	Reference method
Redoxon(Roche)	205.203±9.20	218.02±1.14
Sandoz(Novartis)	129.87±7.72	145.63±2.02

Note: Mean for five determinations

Stability of Vitamin C in Orange Juice

Vitamin C is very susceptible to chemical and enzymatic oxidation during the processing, storage, and cooking of food. The catalyzed oxidation pathway of vitamin C degradation is the most important reaction pathway for the loss of vitamin C in foods. Therefore, vitamin C of orange juice is readily oxidized and lost during staying of the juice (Ball, 2006). On the other hand, there are several factors that will also affect the stability of vitamin C in orange juice. The factors are such as the effect of vitamin E, pH, and parameters which include air, heat, water as well as prolonged storage and overcooking (Kabasakalis, Siopidou, and Moshatou, 2000).

According to Ball, a meta-oxygen-ascorbate complex is formed in the presence of molecular oxygen and trace amounts of transition metal which particularly are copper (II) and iron (III). This complex contains a resonance form of a diradical that rapidly decompose to give the ascorbate radical anion, the original metal ion, and hydrogen peroxide. This radical anion will in turn reacts with the oxygen to give dehydroascorbic acid (DHAA). For anaerobic pathway of vitamin C which occurs in the absence of free oxygen, the degradation is caused by the formation of diketogulconic acid. As the rate of degradation is maximum at pH 3 to pH 4, therefore this pathway is mostly responsible for anaerobic loss of vitamin C in canned grapefruit and orange juices (Ball, 2006) [17].

Effect of Vitamin E on the Stability of Vitamin C in Orange Juice

Vitamin E is a fat soluble antioxidant that has four tocopherols and four tocotrienols. In nature, these four tocopherols and four corresponding tocotrienols are designated as alpha-(?), beta-(?), gamma-(?) and delta-(?) according to the number and position of methyl substituent in chromonal ring (Ball, 2006). The vitamin E functions as a biological antioxidant by protecting the vital phospholipids in cellular and subcellular membranes from peroxidative degeneration. Vitamin E mostly accumulates in body

which are liver and pancreas. But unlike vitamins A and D, vitamin E is essentially nontoxic (Ball, 2006) [18].

Nagymate and Fodor (2008) have designed a method to study the effect of vitamin E on the stability of vitamin C. In this experiment, vitamin E stock solution was prepared by dissolving ?-tocopherol in absolute ethanol. The orange juice which contained vitamin E and vitamin C was used as sample. The storage temperature of the vials was 4° C and they were covered with aluminium foil to prevent the effect of sunlight. Besides, two different temperatures were used to examine the effect of vitamin E at that temperature which half of the samples were stored at 20° C. On the other hand, the additive effect of these vitamins was also examined but only cool samples (4° C) were used for this experiment. Two samples were prepared which one contained vitamin E stock solution and vitamin C stock solution while another contained only vitamin C stock solution. The samples were analysed once a week for five weeks (Nagymate and Fodor, 2008) [19].

The results of the stability of vitamin C show that the presence of vitamin E influenced the decay of vitamin C. Figure 15 shows that there were differences between samples with or without vitamin E. From Figure 15, it can be clearly seen that the concentration of vitamin C without vitamin E fell down to 1.2mg/L on the second day. However, in the presence of both vitamins, the decay was also observed, but it was lesser. The concentration of vitamin C in the orange juice with vitamin E was 13mg/L in the fifth week. As a result, it seems that vitamin E stabilized vitamin C in orange juice at a determined concentration. This is because vitamin E delay the oxidation of vitamin C thus, enhances the stability of vitamin C in orange juice. The combination of vitamin C with vitamin E makes the orange juice more stable and slower the degradation of orange juice. This concluded that orange juice with vitamin E addition is a good way to preserve the vitamin C content during storage (Nagymate and Fodor, 2008).

Effect of Temperature on the Stability of Vitamin C in Orange Juice

Vitamin C of fruit juice is readily oxidized and lost depends on the conditions of storage. There are studies about the determination of the amounts of vitamin C content in fruit juices under different storage conditions. Kabasakalis, Sipadou and Moshatou had done an experiment to determine the rate loss of vitamin C with respect to time and temperature of storage [20]. A long-life and short-life commercial orange juice 100% without preservatives and fresh orange juice were used for analysis. In this experiment, the days before the expiration date were recorded in Table 6 to observe the loss of vitamin C in short-life and long-life orange juice 100% as the expiration date was approached (Kabasakalis, Siopidou and Moshatou, 2000) (Table 6).

Table 6 shows the loss of vitamin C from fresh and long-life commercial orange juice 100% during a 31 days period, with measurements made every 1 to 3 days. The samples were refrigerated into containers which after the initial measurement remained either open or with closed cap until the next measurement. Based on the results shown in Table 5, the magnitude of vitamin C did not differ significantly between open and closed cap for both juices. The commercial orange juice lost higher amounts of vitamin C compared with fresh orange juice. As reported, decreases of vitamin C upon storage did not correspond to increases of dehydroascorbic acid levels. In fact, there was an increase of dehydoascorbic acid levels in aseptically packaged orange juices. This means that the overall nutritional quality of orange juices is affected upon storage (Kabasakalis, Siopidou and Moshatou, 2000).

Table 6: Program of the temperature conditions for sample storage.

Time(h)	Storage Temperature(C)	
	Condition n^0 1	Condition n^0 2
0	4	4
4	8	8
8	4	12
12/72	4	8

The loss of the vitamin C in a commercial long-life orange juice 100% stored in refrigerator and non-refrigerated for a period of 10 days in open containers were shown in Figure 14 (Kabasakalis, Siopidou and Moshatou, 2000). According to Figure 14, non-refrigerated samples show higher percentage loss of vitamin C as compared to refrigerated samples. This is because the dehydoascorbic acid, the oxidized form of ascorbic acid was more stable at lower temperatures. Thus, the vitamin C, in the form of dehydroascorbic acid for refrigerated orange juice was well retained than non-refrigerated orange juice (Kabasakalis, Siopidou and Moshatou, 2000) (Table 7).

Table 7: Assessment of unpasteurized refrigerated orange juice stored under isothermal and non isothermal conditions for72 hours.

Means	Isothermal conditions			Non Isothermal conditions	
	40C	80C	12 0C	$n^0$1	$n^0$2
pH	3.24±0.02	3.27±0.02	3.30±0.02	3.27±0.05	3.28 ±0.03
TSS (0Brix)	10.43±0.36	10.55±0.28	10.54±0.28	10.54±0.28	10.52 ±0.26
TTA (% citric acid)	2.20±0.06	2.27±0.08	2.12±0.04	1.97±0.69	2.02 ±0.13
TSS/ TTA	4.75±0.26	4.88±0.25	4.98±0.15	5.36±0.36	5.22 ±0.35

TSS=total titratable acidity; TTA=total soluble solids; TSS,TTA and pH: mean of the values at time 0,24,48 and72h; condition $n^0$1- 4h/8^{0}C and 60h/4^{0}C; condition $n^0$2-4h/40C, 4h/12^{0}C and 64h/8^{0}C.

Effect of Hydrogen Peroxide on the Stability of Orange Juice

Hydrogen peroxide, H_2O_2 is the primary chemical for sterilization of plastic packaging material used in aseptic system. Aseptic packaging technology is widely used by fruit juice industry for the production of shelf-life stable fruit juices. A Food and Drug Administration (FDA) regulation currently limits the residual of H_2O_2 to 0.5ppm, leached into distilled water, in finished food packages which stated in Code of Federal Regulations, 2000 [21]. However, during the sterilization of aseptic chambers or packaging material with H_2O_2, some residues will still be left on the packaging material or vapors generated during drying may get trapped inside the package upon sealing. These residues will then cause the degradation of vitamin C.

An experiment was proposed by Ozkan, Kirca and Cemeroglu to determine the rates of vitamin C degradation in orange juice with or without addition of H_2O_2 at various storage temperatures. In this experiment, the orange juice sample was thawed at room temperature and sodium benzoate was added to prevent spoilage. The degradation studies were done at H_2O_2 with 0.5ppm concentration at 20°C, 30°C and 40°C respectively. At regular time intervals, samples were removed from the water bath or incubator (Ozkan, Kirca and Cemeroglu, 2004).

Then, the predetermined amounts of diluted sodium hydroxide solution were added rapidly to the samples to halt the reaction between H_2O_2 and vitamin C. The samples were then rapidly cooled by plugging into an ice water bath and held at -30°C until analyzed for vitamin C content. Vitamin C concentration was measured by using HPLC method. Qzkan, Kirca and Cemeroglu had modified the method by blending the orange juice sample with metaphosphoric acid. The sample was filtered through a membrane filter and was analyzed using HPLC (Shimadzu brand) (Ozkan, Kirca and Cemeroglu, 2004).Vitamin C contents of orange juice were plotted for various temperatures at 0.5ppm H_2O_2 concentration.

From Figure 14, the results show that at higher temperature, the rate of vitamin C degradation also increased. The addition of 0.5ppm H_2O_2 did not greatly increase the degradation of vitamin C. However, raising H_2O_2 concentration from 0.5ppm to 5ppm resulted in a tremendous increase in degradation rates which was recorded in Table 6. At 0.5ppm H_2O_2, the antioxidant substances in orange juice which was flavonols reacted with H_2O_2, thereby preventing the autoxidation of vitamin C. The protective mechanism of flavanols was mainly due to chelation of metal ions and action of antioxidant. Flavanols function as antioxidants by donating the hydrogen ions to reactive free radicals which may otherwise cause the autoxidation of vitamin C (Ozkan, Kirca and Cemeroglu, 2004).

Ozkan, Kirca and Cemeroglu also studied the degradation of vitamin C in the absence of H_2O_2. In this case, the activation energy, Ea was taken into account to determine the stability of vitamin C in orange juice. The temperature dependence of the degradation of vitamin C in orange juice was compared by calculating Ea and temperature quotients (Q10) at 20° to 40°C from the following equation: These results clearly indicate that the rate of vitamin C degradation in the presence of H_2O_2 was slower at 30°C to 40°C than 20°C to 30°C. This indicates that at 30°C to 40°C, the least effect of temperature rise on vitamin C degradation.

The results obtained for Ea shows that higher Ea in the presence of H_2O_2. This means that higher energy needed for the degradation of vitamin C. Therefore, the reaction time is slower and the degradation of vitamin C also slower. As conclusion, the effect of temperature on the degradation rates of vitamin C in orange juice was more pronounced at higher H_2O_2 concentrations. Therefore, greater vitamin C losses should be expected as residual H_2O_2 concentration and storage temperature increase in aseptically packaged fruit juices (Ozkan, Kirca and Cemeroglu, 2004) (Figures 14 & 15).

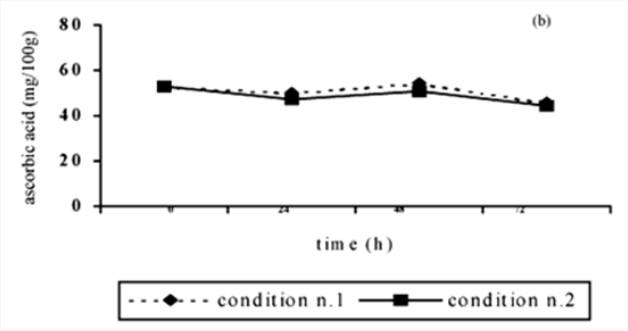

Figure 14: AA Concentration (mg/100g) in natural orange juice stored under isothermal conditions (a) and non-isothermal (condition n^0 2-4h/4^{0}C and 64h/8^{0}C)

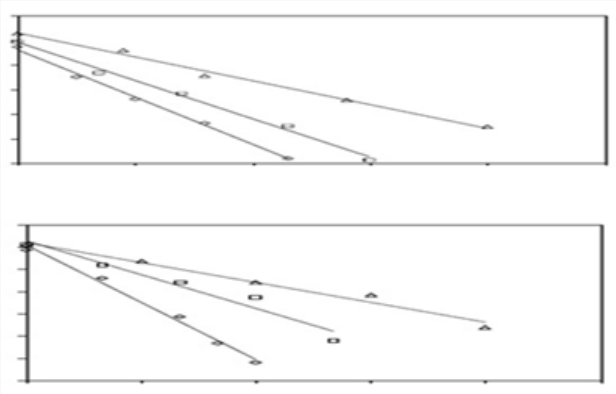

Figure 15

Effect of pH on the Stability of Vitamin C

pH is a measure of acidity or basicity of a solution. pH is one of the primary factor that would affects the stability of vitamin C in orange juice. Hence, the pH value of the matrix has an influence on the stability of vitamin C. According to FAO/WHO Expert Consultation on Human Vitamin and Mineral Requirements, Bangkok, Thailand, 1998, the vitamin C will decay if the pH higher than 4 (Nagymate and Fodor, 2008). Vitamin C is unstable in neutral

and alkaline environments, therefore the higher the pH value and the longer the exposure, the greater the loss of vitamin C. This is because the higher the pH value, the faster the oxidation reaction of vitamin C and causes the degradation of vitamin C. Besides that, the increase in pH also related to deterioration of fruit characteristic which in this literature review, orange juice is more concerned. Table 7 below shows the pH value of the fruit juice with storage time (Ajibola, Babatunde and Suleiman, 2009) (Tables 8 & 9).

Table 8: Reaction rate constants (K) for ascorbic acid degradation in the presence of hydrogen peroxide in various fruit juices during storage.

Sample	H_2O_2 Concentration(ppm)	Temperature(^{0}C)	Zero Order		First Order	
			K(mgl^{-1} h^{-1})	R2	K (h^{-1})	R^2
Orange Juice	0.5	20	1.24	0.978	0.0029	0.922
	0.5	30	2.1	0.953	0.0058	0.854
	0.5	40	3.5	0.989	0.0131	0.885
	5	40	6.62	0.957	0.0184	0.964

In this the pH values of the orange juice were higher at room temperature and keep increasing from week to week. This study concluded that, though pH was significant for the stability of vitamin C, it was not the sole factor in controlling the deterioration of vitamin C in orange juice with storage life (Ajibola, Babatunde and Suleiman, 2009). On the other hand, the loss of vitamin C activity during oxidative degradation of vitamin C occurs with the hydrolysis of the dehydroascorbic acid lactone to yield 2,3-diketogulonic acid. This hydrolysis is favored by alkaline solution. Dehydroascorbic acid is most stable at pH 5.5 but decrease in stability as pH increases which is more than pH 5.5 (Fennmena, 1996). For example, half-time values of dehydroasorbic acid hydrolysis at 23°C were 100 and 230 minutes at pH 7.2 and pH 6.6 respectively.

At pH 5.0 or below, dehydroascorbic acid was quite stable which decayed by less than 3% over 4 hours. This experiment evaluated the effect of hydrogen ion concentration on delactonization of dehyroascorbic acid over the range of pH 3.0 to pH 8.0. The possible influence of the presence of oxygen was done by equilibrating the reaction mixture before and during the incubation with 100% oxygen or with 100% nitrogen. The results indicated no change in the decay rate of dehydoascorbic acid was obvious with these alterations of atmospheric conditions. The rate of dehydroascorbic acid hydrolysis markedly increases with increasing temperature but was unaffected by the presence of oxygen (Bode, Cunningham and Rose, 1990).

Other researchers had proposed a method to determine the effect of pH on the degradation of vitamin C in orange juice. The aim of their experiment was by comparing the stability of vitamin C at different concentrations at lower pH value. An acidic sample was prepared from orange juice with medium alcohol content. The original pH value of sample was later modified by addition of concentrated phosphoric acid. After that, different concentrations of vitamin C stock solutions were added and analysed for five weeks (Nagymate and Fodor, 2008). The results showed that, for the experiment done in original pH value of the orange juice which was pH 4.0, the reduction of the amount of vitamin C content decreased with the increasing ascorbic acid concentration ($p> 0.05$), so the speed of decay was higher at lower concentrations. In the case of orange juice, the highest standard deviation of the repeated data was 3.14% and the lowest standard deviation was 1.48%. This indicates that the results are accurate and reproducible (Nagymate and Fodor, 2008).

Table 9: Natural Orange Juice Ascorbic acid content (mg/100mL) at different storage times after squeezing.

Time	Mean±σ(% Retention)	
	1	2
0	55.26±0.22 (100)	50.54±0.15 (100)
1	53.85±0.14 (97.45)	-
3	52.40±0.33 (94.82)	50.36±2.44 (99.64)
5	50.76±0.97 (91.86)	-
6	50.00±0.97 (90.48)	50.33±0.47 (99.58)
24	48.20±3.27 (87.22)	50.22±0.60 (99.45)

When lower pH (pH 3.0) was used, the speed of decay for orange juice grew with the growing vitamin C concentration, and the highest value was at 50mg/L vitamin C concentration ($p> 0.05$). These results were tabulated in Table 9. The highest standard deviation of the repeated data was 3.42% and the lowest standard deviation was 1.53%. By comparing the statistical data in both Tables 8 & 9, it shows that the lower pH values increased the vitamin C content measured at the end of fifth week (Nagymate and Fodor, 2008). According to Nagymate and Fodor, vitamin C had a significant decay independently from the storage temperature when the pH value was more than 4.0. Nevertheless, under this pH limit, low storage temperature will help in stabilizing this vitamin. Hence, lower pH value was preferred to prolong the shelf life of orange juice.

Materials and Methods

Analysis of Vitamin C Content in Vitamin C Tablets

Instrument, Materials and Chemicals

The instrument that used in this research is Reflectometer.

The chemicals that used in this research are as below

a) 1 gdm-3 of Ascorbic Acid (Sigma)

b) 0.1M Sodium Hydroxide (R & M Chemicals)

c) Phenolphthalein indicator

d) Potassium acid phthalate (Hamburg Chemical GMBH)

8.2. The materials that used in this research are as below

a) Flavettes Vitamin C-Sugar-Free C

b) Redoxon Orange

c) Cebion Vitamin C

d) Bio C Plus (Anway)

Product Sample Preparation

The vitamin C tablets were crushed into powder by using mortar and pestle. 1gdm-3 of each sample was prepared by dissolving 0.1±0.05g of the samples with distilled water and top up to 100mL. The solutions were thoroughly mixed. The solutions were kept away from direct sunlight and stopper was used to minimize the oxidation of ascorbic acid.

Reagent Solution Preparation

Preparation of Vitamin C Standard Solution

A freshly prepared ascorbic acid solution was prepared by dissolving 0.02g of ascorbic acid and top up to 100mL with distilled water in a volumetric flask. The solution was thoroughly mixed. The solution was kept away from direct sunlight and a stopper was used to minimize the oxidation of ascorbic acid.

Preparation of Sodium Hydroxide Solution

0.1M of sodium hydroxide solution was prepared by adding 2.0g of sodium hydroxide (NaOH) pellets and was topped up to 500mL into a volumetric flask. This solution was mixed and was standardized using potassium acid phthalate (KHP) solution.

Experimental Procedure

Determination of Vitamin C by using Reflectometer (MERCK)

As control method for ascorbic acid test, 0.02g of ascorbic acid was diluted with 100mL of distilled water. The ascorbic acid solution should be freshly prepared. Beside that, two additional tests were done by using the same instrument but different test kits which used to test the glucose and Hydroxymethylfurfural (HMF) test. The respective strip was immersed into the sample for few seconds. The test strip was inserted into the strip adapter. At the end of reaction time, the result was read from the display.

Determination of Vitamin C by using Titration Method

Standardization of 0.1M Sodium Hydroxide (NaOH): Standard potassium acid phthalate (KHP), $KC_8H_4O_4H$ solution was prepared by weighing 0.5g dried KHP into an Erlenmeyer flasks and was dissolved by adding 75mL of distilled water. The molecular weight of KHP is 204.23. Three drops of phenolphthalein indicator were added into each Erlenmeyer flask. For 0.1M NaOH standardization, KHP solution was used and titrated with 0.1M NaOH until the faintest pink persists for 30 seconds. The final volume was recorded. The standardization of NaOH was repeated for another two times.

Vitamin C Analysis: Quantitative Method

0.2g of vitamin C was weighed and then added into an Erlenmeyer flask. 50mL of distilled water was added to dissolve the vitamin C tablet. Three drops of phenolphthalein indicator were added into the Erlenmeyer flask. A burette which contains 0.1M of NaOH solution was set up as which as shown in Figure 15. The vitamin C solution was titrated with NaOH solution was until a pink color that persists for 30 seconds which was the end point. The final volume was recorded. These procedures were repeated for another two sets of sample.

Result and Discussion

Determination of Vitamin C by using Reflectometer (MERCK)

In this project, reflectometer and titration method were used to determine the vitamin C content in four pharmaceutical products which are Flavette, Cebion, Bio C Plus and Redoxon Orange. In reflectoquant analysis, Reflectoquant Test Strips are inserted into the instrument (RQflex 10) which is a highly sensitive reflectrometry instrument. The combination of these two tests which are the test strips and the reflectometer help to analyze the vitamin C content for a wide variety of samples in just few seconds. Reflectometer uses a double optic system which works in conjunction with a dual reaction zone on Reflectoquant Test Strips to allow for simultaneous double measurements in one step. It is a portable test system that is small, compact, and battery-operated for rapid, quantitative analysis of various samples by evaluation of special test strips. Vitamin C contents in each sample which is determined using reflectometer do not have much different compared to the label value. Tablets are labeled according to their vitamin C content and not according to their weight.

The percentage of deviation of each sample was calculated and the percentages of deviation obtained for all the samples are less than 5.0%. This indicates that reflectometer can provide accurate and reproducible results. The difference between label value and analysis results could be caused by the interference substances such as the presence of binder. Binders are commonly used when making conventional tablets. Most binders are polymers which can increase the plastic deformation of the formulation. Binder can be used to prevent a rapid dissolution of the effervescent tablet such as Redoxon. Examples of binders are such as methyl cellulose and gelatin which function to hold the ingredients together to form a tablet (Swarbrick and Boylan, 1992). For Redoxon, the analysis result is lower than the label value. This is due to the presence of foreign substances such as zinc citrate which may influence the concentration of vitamin C.

Determination of Glucose Content and Hydroxymethylfurfural Content in Vitamin C Tablet

Glucose content in each tablet of different brands is less than 1g/mL except for Flavette Vitamin C. Flavette Vitamin C contains 22mg/L of glucose content although this produc is labeled sugar free. Many manufactures use glucose, fructose or dextrose to sweeten a tablet for commercial purpose even though the tablet labeled 'no sugar'. For hydroxymethylfurfural (HMF) test, 5-(Hydroxymethyl) furfural (5-hydroxymethyl-2-furancarbaldehyde, HMF) reacts with a barbituric derivative and an aminophenazone derivative to form a red-violet compound that is determined reflectrometrically.

HMF test was done to test the amount of undesirable product such as 5-HMF in vitamin C tablet. From the results, all the product samples contain about 1.3 to 1.5 mg/L of HMF. Based on the Commission Regulation, the average usage of HMF is 2.0mg/L and the maximum usage is 10.0mg/L for non-alcoholic products (Berger, 2007). HMF is a crystalline product with a pleasant odor. HMF is formed in foods by thermal treatment during storage (Nollet, 2004). High amount of HMF can cause vitamin C loss, hence affecting the quality of the product (Damasceno et al, 2008) [22].

Determination of Vitamin C by using Titration Method

Standardization of Sodium Hydroxide (NaOH) solution using potassium acid phthalate solution (KHP)

In a titration, it is critical to know the exact concentration of NaOH in order to determine the concentration of the solution being tested. KHP is a weak acid and reacts with base in the following way:

In the titration method, phenolphthalein was used as an indicator which will be used to determine when the reaction reaches its endpoint. Endpoint is the point which the amount of NaOH added equals the amount of vitamin C. By knowing the strength and the volume of NaOH required to completely react with vitamin C, the actual amount of vitamin C present can be calculated. pH meter also helps to determine the end point which is about pH8.50. As vitamin C is a weak acid, the pH of the end point is detected by using phenolphthalein indicator with the transition range between pH8.

0-9.8. Phenolphthalein will changes from colorless to pink when all of the acid has been neutralized. The samples were analyzed by the reflectometer and the titration methods are summarized in Table 7. The results show that both methods are in agreement with the quantities specified on the label. This indicates that the proposed method was applied successfully for the determination of vitamin C in commercial pharmaceutical products.

Conclusion

Vitamin C is required for the optimal activity of several important biosynthetic enzymes and it is therefore essential for various metabolic pathways in the body. However, according to RDA for vitamin C, 75mg/day and 90mg/day are required for normal women and men respectively. This level is believed sufficient enough to prevent deficiency disease but not chronic disease. Owing to this, vitamin C should be taken each day to prevent chronic disease and the effective doses are still remained unclear today. On the other hand, the Tolerable Upper Intake Level (UL) is 2000mg/day where too much of vitamin C may be dangerous due to the adverse effects such as kidney stone formation, increase of uric acid excretion and overload iron.

There are few factors that will affect the stability of vitamin C which are vitamin E, pH, amount of hydrogen peroxide and temperature. Additionally, freshly prepared orange juice should be taken in as soon as possible. This because vitamin C may be oxidized during storage even we store it in refrigerator. It was found that vitamin C loss is the most when the orange juice was stored at room temperature. Well-pasteurized package juice from market can lose its vitamin C as well due to long storage time even if it is not opened. The highest loss of vitamin C occurred with conventional boiling. The research also shows that orange juice with vitamin E can delay the degradation rate of vitamin C. Besides that, high concentration of hydrogen peroxide will cause greater loss of vitamin C. Finally, lower pH value was preferred to prolong the shelf life of orange juice.

Three different methods are studied in the review part which is flow-injection analysis, ultraviolet spectrophotometry and fluorometric method. All these methods are using spectrophotometer as the detector to determine the content of vitamin C in a sample. Nevertheless, all these methods required highly cost equipment and reagent in order to perform an analysis than reflectometric method. In my project, the proposed method using reflectometer can provide a simple analysis of vitamin C. The simplicity of the procedure permits rapid analysis for vitamin C content in pharmaceutical products. This method is found to be more sensitive and reliable. Besides that, the time required for sample preparation is short and reagent consumption is also low, hence this method is highly economical.

In addition, by using reflectometer, it is a good alternative method compared to some of the highly cost instrument method. Therefore, it is suitable to use on routine basis for the determination of vitamin C in pharmaceutical preparations. It is desirable that nutrition education should be brought into the public understanding of science. Moreover, more researches should be done on the field of the relation between diseases and vitamin C intake. Food or crop engineering is another critical field in the study of vitamin C where this can improve the concentration of ascorbic acid in natural food.

References

1. Levine M, KR Dhariwal, RW Welch, Y Wang, JB Park (1995) Determination of optimal vitamin C requirements in humans. Am J Clin Nutr 62: 1347S-1356S.

2. Lidija B, L Pfendt, LV Vesna, BZ Nada, RP Mirjana (2003) Second order derivative spectrophotometric method for determination of vitamin C content in fruits, vegetables and fruit juices. Eur Food Res Technol 217(3): 269-272.

3. Alam MA (1996) Comparative study of total vitamin C in various fruits and vegetables of greater Sylhet area. MSc Thesis, SUST, Sylhet.

4. Albert LL (1993) Principles of Biochemistry. CBS Publishers and Distribution Pvt Ltd, New Delhi.

5. Bendich A (1997) Vitamin C Safety in Humans. In: Vitamin C in Health and Disease, Packer, L and J. Fuchs (Eds.); Marcel Dekker Inc New York, USA, pp: 367-379.

6. Block G (1991) Epidemiologic evidence regarding vitamin C and cancer. Am J Clin Nut 54: 1310S-1314S.

7. Block G (1992) The data support a role for antioxidants in reducing cancer risk. Nutr Rev 50(7): 207-213.

8. Frei B (1994) Reactive oxygen species and antioxidant vitamins: Mechanism of action. Am J Med 97(3A): 5S-13S.

9. Geigertj J, DS Hirano, SL Neidleman (1981) High-performance liquid chromatographic method for the determination of L-ascorbic acid and D-isoascorbic acid. J Chromatogr 206(2): 396-399.

10. Groff JL, S Groppers, SM Hunt (1995) The Water Soluble Vitamins in Advanced Nutrition and Human Metabolism. West Publishing Co, Minneapolis, Belmont, pp: 222-237.

11. Indian Pharmacopoeia (1985) A Facile and Sensitive Spectrophotometric Determination of Ascorbic acid. Talanta 34(8): 731-732.

12. Veasey RL, TA Nieman (1980) Chemiluminescence high-performance liquid chromatographic detector applied to ascorbic acid determinations. J Chromatogr 200: 153-162.

13. Weber P, A Bendich, W Schalch (1996) Vitamin C and human health-a review of recent data relevant to human requirements. Int J Vitam Nutr Res 66(1): 19-30.

14. Woodall AA, BN Ames (1997) Diet and Oxidative Damage to DNA: The Importance of Ascorbate as an Antioxidant. In: Vitamin C in Health and Disease. Packer L, J Fuchs (Eds.); Marcel Dekker Inc, New York, pp: 193-203.

15. Jacobs MM (1993) Diet, nutrition, and cancer research: An overview. Nutr Today, pp: 19-23.

16. Jaffe GM (1984) Vitamin C. In: Handbook of Vitamins, Machalinal L Mercell Dekker Inc New York, pp: 199-244.

17. Khan MM, MM Rahman, ATM Murad, SA Begum (2005) Determination of vitamin C content in various fruits and vegetables by UV-spectrophotometric method at Sylhet area, Bangladesh. J Environ Sci 11: 190-193.

18. Levine M (1986) New concepts in the biology and biochemisty of ascorbic acid. New England. J Med 314(14): 892-902.

19. Levine M, Conry-Cantilena, Y Wang, RW Welch, PW Washko, et al. (1996) Vitamin C pharmacokinetics in healthy volunteers: Evidence for a recommended dietary allowance. Proc Natl Acad Sci USA 93(8): 3704-3709.

20. Rath M (1993) Eradicating Heart Disease. Health Now, San Francisco, USA.

21. Riemschneider R, MZ Abedin, RP Mocellin (1976) Qualitats and stabilisierungprufung hitzekonservierter Nahrungsmittel unter verwendung von Vit C als kriterium-Mitt 1. Alimenta 15: 171-171.

22. Sies H, S Wilhelm (1995) Vitamins E and C, Beta-carotene, and other carotenoids as antioxidants. Am J Clin Nutr 62(6): 1315S-1321S.