CHAPTER 1

INTRODUCTION

1.1 Vitamin C

Vitamin C (ascorbic acid) is a vitamin soluble in water and is not synthesized by humans' bodies, but it is an essential nutrient [1]. It is important in processes of metabolic reactions such as amino acid metabolism, increasing the absorption of iron and antioxidation activity. Vitamin C deficiency causes scurvy and symptoms such as gingivitis, susceptibility of blood vessels to damage and retarded wound healing [2]. Vitamin C can be synthesized from glucose and its chemical structure $C_6H_8O_6$ is shown in Figure 1.1

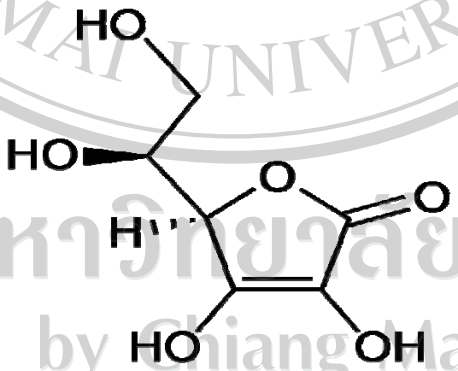

Figure 1.1 The structure of vitamin C (L-ascorbic acid) [3]

Vitamin C has a molecular weight of 176.13 and is a white crystalline. The melting point is 190-192 °C. The natural form of the vitamin C is L-ascorbic acid; it can be reversible oxidized to dehydroascorbic acid, forming a useful redox system [4, 5].

Vitamin C is present naturally in a wide range of vegetables and fruits such as tomatoes, lemons, strawberries, oranges and pineapples. However, vitamin C has limited stability which is easily destroyed by cooking and by contacting with alkaline substances. Some vitamin C is lost in freezing and preserving, and it is also easily oxidized [6].

1.2 Analytical methods

Many analytical methodologies including flow injection system have been proposed for determination of vitamin C. Examples of these methods are shown below:

1.2.1 Titrimetric methods

Several titrimetric methods for determination of ascorbic acid based on oxidation-reduction reaction have been reported [4]. The 2,6-Dichlorophenol indophenol is popularly used. It is reduced by ascorbic acid, changing from the blue color of the oxidized dye to a colorless solution [6]. Ascorbic acid is oxidized to dehydroascorbic acid and excess dye remains pink in acid solution, forming the visual endpoint of the titration (colorless to pink). Other reagent such as N-Bromo-succinimide [3, 8] has also been used for the assay of ascorbic acid. Furthermore, method has been applied for determination of ascorbic acid using iodide and a starch

indicator. The iodine reacts with ascorbic acid and in excess, forming a blue-black complexes with indicates the end-point of the titration.

1.2.2 Electroanalytical methods

These methods are mainly based on the inherent redox chemistry of the analyte and reagents. The photochemical reduction of methylene blue and the redox reaction of iodate ion were employed in amperometric determination [5, 8]. In addition, many methods have been applied for determination of ascorbic acid such as voltammetric, square wave voltammetric, potentiometric, polalographic and coulometric methods [10-15].

1.2.3 Chemiluminescence methods

Chemiluminescent methods have been proposed [5]. This methods involved the oxidation of ascorbic acid by iron(III), hexacyanoferrate(III) or permanganate [16] followed by the interaction with another chemiluminescent reagent such as luminal [8].

1.2.4 Fluorimetric methods

These methods are based on the formation of fluorescent species in redox reactions where oxidation of ascorbic acid is produced [10]. The formation of fluorescent product by the reaction of dehydroascorbic acid with *o*-phenylenediamine is the basic of many methods, including the AOAC official method [4, 8]. Furthermore, method has been applied for determination of ascorbic acid using β-cyclodextrin derivative [17]. The fluorescence intensity of β-cyclodextrin derivative decreases as

the mono-[6-N(4-carboxy-phenyl)]-cyclodextrin-ascorbic acid supramolecular complexes are formed.

1.2.5 Chromatographic methods

Chromatographic methods allow the determination of the combined physiologically active forms of vitamin C that is ascorbic acid and dehydroascorbic acid [18-22]. Several HPLC methods have been developed for the analysis of vitamin C. These assays utilized various column packings and solvent compositions, and detection is based on the UV absorbance or the electrochemical properties of vitamin C [23]. For example, the HPLC is conducted after dehydroascorbic acid reduction by L-cysteine or dithiothreitol or derivatized with *o*-phenylenediamine to form the fluorophore 3 (1,2-dihydroxyethyl) furo [3,4-b] quinoxaline-1-one [24-25]. Other methods are based on electrochemical, voltammetric, chemiluminescence and fluorometric detection [18, 23].

1.2.6 Spectrophotometric methods

Spectrophotometric method is more frequently used. These methods involve redox reactions with ascorbic acid [5]. The methods using chloramines T in the presence of starch-KI solution, the formation of Fe(II)-phenanthroline, Fe(II)-ferrocine or Cu(I)-bathocuproine complexes prior to metal ions reduction by ascorbic acid have been reported [26-27]. Measurement of decrease in color intensity was also employed by reduction of cerium(IV), triiodide, Co(III)-EDTA complex or photochemical reduction of methylene blue [8]. There have been many methods proposed for

determination of ascorbic acid by spectrophotometry and many flow injection procedures have been proposed. One example investigated the reduction of ascorbic acid, using the fact that the color of potassium permanganate is decreased by the redox reaction [28]. The change of color intensity can be monitored by using spectrophotometry.

1.2.7 Miscellaneous methods

Some techniques have been used for ascorbic acid assay such as ^{13}C NMR or PMR spectrometry or ESR spectrometry and immunobiosensor [8, 29-30].

Flow based analysis such as flow injection analysis, stopped-flow injection analysis and sequential injection analysis is now a widely used technique for determination of ascorbic acid [31-45]. Stopped-flow injection analysis is used for increasing sensitivity for trace ascorbic acid. Some example of flow injection analysis and stopped-flow injection analysis over the period 1995-2004 are summarized in Table 1.1

Table 1.1 Summarization of flow analytical methods for determination of vitamin C

Techniques	Sample	Conditions	Analytical characteristic	Ref.
Flow injection spectrophotometer	Vitamin C tablet	Vitamin C was reacted with permanganate or ammonia. The absorbance was monitor at $\lambda = 302$ nm.	% RSD = 0.5 Sample rate of 90 s/h	[34]
Flow injection amperometer	Beverage	The amperometric detection was performed at 0.5 V. versus Ag/AgCl/NaCl (sat.)	Working range 4.7×10^{-7} - 1.0×10^{-3} M DL = 4.7×10^{-7} M	[35]
Flow injection spectrophotometer	Pharmaceutical sample, oranges and tomatoes	Vitamin C was reacted with potassium dichromate-potassium iodide/ rhodamine.	Working range 0.10-4.00 μg/ml DL = 0.08 μg/ml	[36]

Table 1.1 (Continue)

Techniques	Sample	Conditions	Analytical characteristic	Ref.
Flow injection spectrophotometer	Pharmaceuticals	The reduction of mono (1,10-Phenanthroline)-iron (III) complex was carried out by reducing action of dienol group of ascorbic acid. The absorbance of the resultant tris chelate was monitored at 510 nm.	Working range 0-50 mg/L % RSD = 0.5 Sample rate of 200 s/h	[37]
Flow injection spectrophotometer	Fruits and vegetables	Bathophenanthroline disulphonic acid – iron (III) was used as an oxidant at room temperature	Working range 1-10 μg/ml Sample rate of 80 s/h	[38]
Flow injection cyclic voltammeter	Urine	The anodic oxidation of ascorbic acid was carried out on a ruthenium oxide hexacyanoferrate modified electrode at pH 6.9.	% RSD = 2.0 DL = 2.2 μM	[39]

Table 1.1 Summarization of stopped-flow analytical methods for determination of vitamin C

Techniques	Sample	Conditions	Analytical characteristic	Ref.
Stopped-flow spectrofluorimeter	Pharmaceutical, wine, beer and urine	The enzymatic kinetic reaction of vitamin C with *o*-phenylenediamine was carried out. The variation of fluorescence at λ_{em} = 430 nm (λ_{em} =360 nm) was monitored.	Working range 0.025 - 1.0 μg/ml	[40]
Stopped-flow spectrophotometer	Kinetic of ascorbic acid	Kinetic of ascorbic acid was investigation by peroxynitrite. The absorbance was monitor at λ = 302 nm	DL = 0.3 mM	[41]
Stopped-flow spectrophotometer	Ascorbic acid	Kinetic investigation of the oxidation of ascorbic acid by Iron (III) benzohydroxamic acid was done in ethanol medium at pH values.	-	[42]

Table 1.1 (Continue)

Techniques	Sample	Conditions	Analytical characteristic	Ref.
Stopped-flow spectrofluorimeter	Pharmaceutical	Vitamin C was reacted with *o*-phenylenediamine. The fluorescence was monitor at λ_{em} = 530 nm (λ_{em} = 430 nm).	Working range 0.02-2.0 μg/ml DL = 0.014 μg/ml	[43]
Stopped-flow spectrofluorimeter	Fruit juices, soft drinks and blood serum	Ascorbic acid was oxidized by dissolved oxygen to dehydroascorbic acid, which then reacts with *o*-phenylenediamine to form a fluorescent quinoxaline.	Linear range = 0.1–30 μg/ml % RSD = 0.5	[44]
Stopped-flow amperometer	Pharmaceutical	Vitamin C was reacted with *p*-benzoquinone to hydroquinone. The maximum amperage of 7.5 A was observed.	DL = 6 nM	[45]

The table indicates that the stopped-flow system can be combined with various detection methods. Spectrophotometric method is popularly used for determination of vitamin C. Many reactions have been applied using conventional batch methods, which are tedious, time consuming and high reagent volume consumption. The stopped-flow system can be used to increase the sensitivity and reliability of the measurement for determination of vitamin C.

1.3 Stopped-flow injection technique

Stopped-flow is one of the flow injection techniques that increases reaction time and decreases dispersion of product zone, and hence increases sensitivity [46-49]. Sensitivity for the determination can be increased when stopping the reaction mixture in a stopping coil. After a desired period of time, the product zone is moved to a detector. In this mode, the height or area of the signal can be used for measurement. General stopped-FI gram is illustrated in Figure 1.2

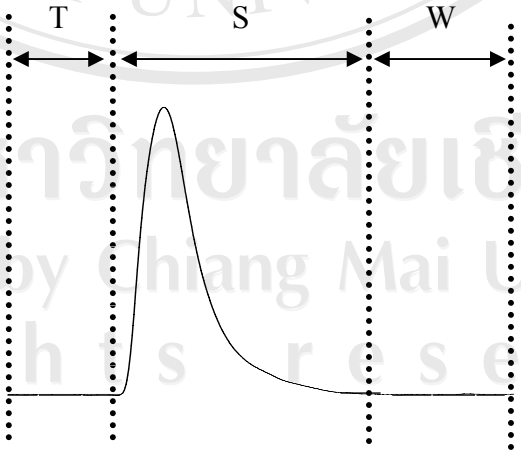

Figure 1.2 General stopped-FI gram: T = Travelling time, S = Stopping time, W = Washing time.

From Figure 1.2, travelling time (T) is the time duration that the standard and reagent combine while flowing before being stopped in the mixing coil. Stopping time (S) is the period of time during the mixture is stopped in the stopping coil. Washing time (W) is the time duration where the mixture is restarted to flow (after stopping).

Stopped-flow system has been developed to minimize reagent consumption. This system was applied for the assay of vitamin C, using sodium molybdate as a reagent and the spectrophotometer as a detector. The reaction is based on molybdenum blue method [47]. The sensitivity can be increased by increasing the stopped time.

1.4 Research aims

This research work aims to develop the cost-effective stopped-flow system and low reagent consumption for determination of vitamin C in some fruits using molybdenum blue reaction.