Chiang Mai J. Sci. 2008; 35(2) : 345-354
www.science.cmu.ac.th/journal-science/josci.html
Contributed Paper

Flow Injection Amperometric Method with Dialysis Sample Pretreatment for Determination of Ascorbic Acid

Pipoon Bunpeng [a], Somchai Lapanantnoppakhun [a,b], and Jaroon Jakmunee *[a,b]

[a] Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai, 50200, Thailand.

[b] Institute for Science and Technology Research and Development, Chiang Mai University, Chiang Mai, 50200, Thailand.

*Author for correspondence; e-mail: scijjkmn@chiangmai.ac.th

Received: 20 December 2007
Accepted: 24 January 2008.

ABSTRACT

A flow injection amperometric method with dialysis sample pretreatment for determination of ascorbic acid (vitamin C) has been developed. Standard/sample solution (50 μL) was injected into a donor stream (deionized water) via an injection valve. The sample zone was passed through a dialysis unit to separate the analyte from the matrices. The dialysate containing ascorbic acid, in the acceptor stream of 0.1 M phosphate buffer pH 5.6, was flowed to the amperometric detector for current measurement. Ascorbic acid was electrochemically oxidized at glassy carbon electrode giving an anodic peak current proportional to ascorbic acid concentration. A linear calibration graph in range of 50 - 800 mg/L ascorbic acid was obtained. The relative standard deviation of 10 replicate injections was 1.5 % for 50 mg/L of ascorbic acid. A sample throughput of 20 h^{-1} was achieved. The proposed method was applied to vitamin C tablets and fruit juice samples collected from drug stores, supermarkets and local suppliers in Chiang Mai. The results obtained for vitamin C samples are agreed well with the labeled values, and comparable with those determined by voltammetric method. The proposed method is rapid, low reagent consumption and does not suffer from colored and colloidal substances presented in the sample.

Keywords: flow injection, amperometry, dialysis, ascorbic acid, vitamin C, fruit juice.

1. INTRODUCTION

Ascorbic acid (vitamin C), a water soluble vitamin is an important micronutrient and plays many physiological roles in the body, including as a free radical scavenger, which may help to prevent free radical induced diseases such as cancer and Parkinson's diseases[1]. It has also been used for the prevention and treatment of the common cold, mental illness, infertility, cancer and AIDS[2]. Fruits and vegetables are the principal source of ascorbic acid. Ascorbic acid is also added into food and beverage products and pharmaceutical preparations as a supplementary source of vitamin C in human

diets. However, ascorbic acid is not stable in the presence of air, heat, light and some metal ions. It is necessary for industry to have rapid and sensitive methods for routine and reliable determination of ascorbic acid. Although method based on visual end point indophenols titration has been approved by the Association of Official Analytical Chemist (AOAC) for the determination of ascorbic acid in food products, it has several drawbacks such as it is tedious and time consuming procedure with high chemical consumption, and colored species in sample may interfere a lot on end point detection. Various instrumental methods have been proposed including spectrophotometry[3], chemiluminescence[4], enzymatic analysis[5], high performance liquid chromatography[6], electrophoresis[7] and electrochemistry[8-9].

Many flow injection (FI) methods have been reviewed for the determination of ascorbic acid in pharmaceutical preparations, food products, and biological samples using different detection systems [10]. Most of the methods employed the reducing property of ascorbic acid. Spectrophotometry in visible region is the technique more frequently used. It involved redox reaction with ascorbic acid in which a colored compound is formed or decomposed in a reaction. For example, the method using chloramine T in the presence of starch-KI solution[11], the titration with 2,6-dichlorophenolindophenol[12], the formation of Fe(II)-phenanthroline[13], Fe(II)-ferrocine[14] or Cu(I)-bathocuproine [15] complexes previous metal ions reduction by ascorbic acid have been reported. Decolorized methods such as by reduction of cerium(IV)[16], reduction of triiodide [17], reduction of Co(III)-EDTA complex [18] or photochemical reduction of methylene blue[19], and a simple fading of permanganate color by ascorbic acid[20] were proposed. Colored substances presented in sample may seriously interfere in these methods. On the other hand, electrochemical technique did not suffer from this kind of interference. The technique is based on direct electrochemical oxidation of ascorbic acid on a bare glassy carbon (GCE) or platinum (Pt) electrode or other modified electrodes. Electrooxidation on bare GCE or Pt electrode requires higher potential than the modified electrode, which may lead to electrode fouling, poor reproducibility and low selectivity, especially in batch procedure[21]. Modified electrodes have been proposed to avoid these problems [21,22], but complicated process in preparation and limited stability of the electrode were found.

In this work, FI amperometric system using a bare GCE as a working electrode was developed for determination of ascorbic acid. With injecting small volume of sample and incorporating of on-line dialysis unit, reproduce signal with improving in selectivity and without electrode fouling was obtained. The dialysis unit also provided on-line dilution of sample, which percentage dialysis of 0.3% was achieved for cellulose acetate membrane with molecular weight cut off of 12,000 Da. Although a lab-built amperometer with a home-made data acquisition device was employed, a good sensitivity and linearity calibration graph was obtained in range of 50-800 mg/L ascorbic acid. The proposed method was applied for pharmaceutical and fruit juice samples.

2. MATERIALS AND METHODS

2.1 Chemicals and Samples

All solutions were prepared with analytical grade chemicals and with deionized water. Standard solution of ascorbic acid (1000 mg/L) was prepared daily by dissolving 0.1000 g of ascorbic acid (UNILAB) in water and adjusting to 100 mL in a volumetric flask. Supporting electrolyte (0.1 M phosphate

buffer of pH 6) was prepared by dissolving 14.71 g of sodium dihydrogenphosphate dihydrate(Fluka) and 1.00 g of disodium hydrogenphosphate dihydrate(BDH) in water and making up volume to 500 mL.

Pharmaceutical tablet samples were obtained from local pharmaceutical stores. Twenty tablets of the sample were weighed and an average weight of a tablet was calculated before being ground into fine powder. Then, 0.6-4.0 g portions were accurately weighed, dissolved in water to get appropriate concentrations of ascorbic acid in solutions.

Fruit juice samples were collected from supermarkets and local suppliers. Then, the samples were filtered with cotton wool before analyses.

2.2 FI Manifold and Procedure

Schematic diagram of the instrumental set-up of FI amperometric system is shown in Figure 1. It consisted of a peristaltic pump (Ismatec, Switzerland), a six port injection valve (Upchurch, USA), a home-made dialysis unit[23] and a lab-built amperometer with a flow through electrochemical cell (BAS, USA) and an in-house developed data recording unit[24] or a commercial data acquisition system(eDAQ, Australia). The planar dialysis unit was similar to the previous report[23], but with a dialysis membrane (cellulose acetate membrane of MW cut off 12,000 Da) instead of a Teflon membrane. A GCE (3 mm diameter), stanless steel and Ag/AgCl electrodes were used as working, auxiliary and reference electrodes, respectively. Standard or sample (50 μL) was injected into a water carrier stream and flowed to dialysis unit, where ascorbic acid dialysed to an acceptor stream, 0.1 M phosphate buffer pH 5.6. Flow rates of both the donor and acceptor streams are 1.5 mL/min. Ascorbic acid was diluted and separated from other matrices in the samples. A zone of ascorbic acid solution in the acceptor stream flowed further to the flow through electrochemical cell, where a constant potential (0.8 V) was applied to a GCE. Electrooxidation of ascorbic acid produced anodic peak current which was converted to voltage and recorded as FI peak. A calibration graph is a plot of peak height versus ascorbic acid concentration. Concentration of ascorbic acid in sample was calculated using an equation obtained from a linear calibration graph.

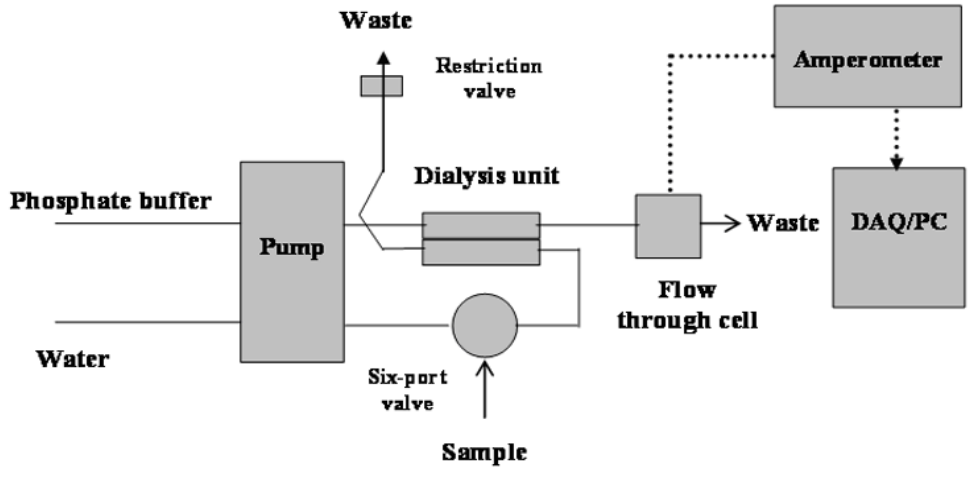

Figure 1. Schematic diagram of the instrumental set-up of FI amperometric system with on-line dialysis.

3. RESULTS AND DISCUSSION

3.1 Optimization of the FI Amperometric Detection System

Using a single line FI – system without dialysis unit, 50 μL of the ascorbic acid standard solution was introduced into phosphate buffer carrier stream via a six-port valve. The injected solution flowed through the flow cell whereas ascorbic acid was oxidized to dehydroascorbic acid at a working electrode. Preliminary conditions, 0.1 M phosphate buffer solution of pH 6.0 as a carrier solution, flow rate of 1.0 mL/min were employed. Effects of various parameters were investigated as followed.

3.1.1 Type of Working Electrode

Two types of working electrode, platinum disc (2 mm diameter) and glassy carbon disc (3 mm diameter) electrodes were investigated, with a fixed potential of 0.1 V applying to the electrode. A standard solution of 100 mg/L ascorbic acid was injected in 11 replicates into the system. The peak heights of 19.2±0.6 mV and 20.5±0.6 mV were obtained for Pt and GC electrodes, respectively. Despite both of the electrodes could be used, the GCE was selected for further experiment.

3.1.2 Applied Potential

The applied potential was varied in the range of 0.1-1.4 V vs. Ag/AgCl reference electrode. A 40 mg/L ascorbic acid standard solution was injected into the system. It was found that peak current obtained increased sharply with applied potential in the range of 0.1-0.8 V and reached maximum at 1.0 V (Figure 2). However, baseline drift was observed for applied potential higher than 0.8 V and higher potential may lead to oxidation of some interfering substances, so applied potential of 0.8 V was selected.

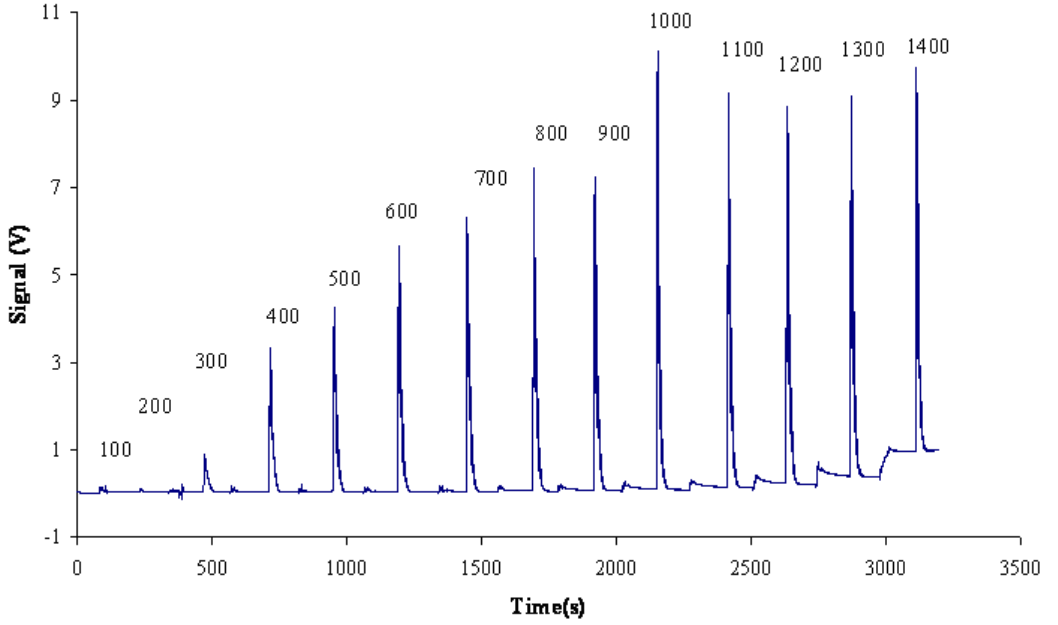

Figure 2. FIgram obtained from injecting of 40 mg/L ascorbic acid with different applied potentials (mV) to the GCE.

3.1.3 Concentration and pH of Phosphate Buffer

Concentration of phosphate buffer in the range of 0.005 – 0.5 M was investigated, while pH of buffer was fixed at 6.0. Calibration graphs in concentration range of 20 – 100 mg/L ascorbic acid were constructed by plotting peak height obtained versus ascorbic acid concentration. Slopes, intercepts and r^2 of the calibration graphs are shown in Table 1. Phosphate buffer concentration of 0.1 M was chosen because it provided high sensitivity with using smaller amounts of reagent than 0.5 M.

The pH of phosphate buffer was varied from 3.0 -11.0, while phosphate buffer concentration was fixed at 0.1 M. Standard solutions of ascorbic acid in concentration range of 20 – 100 mg/L were injected. With increasing of the pH, slope of the calibration graphs slightly decreased as shown in Table 2. Therefore, buffer of pH 5.6 was chosen because this solution gave high sensitivity and was easy to prepare.

3.1.4 Flow Rate

Effect of flow rate of phosphate buffer carrier solution (0.5 – 4.8 mL/min) was investigated by injecting various concentrations of ascorbic acid standard solution (20 -100 mg/L) and constructing a calibration graph for each flow rate. Slope of the calibration graphs are plotted versus flow rate of the phosphate buffer, as illustrated in Figure 3. It was found that the higher flow rate of the carrier stream, the higher sensitivity was

Table 1. Effect of concentration of phosphate buffer on the determination of ascorbic acid by FI amperometric method.

Concentration of phosphate buffer (M)	Calibration data		
	Slope (mV.L/mg)	Y – intercept (mV)	r^2
0.005	1.0	164.9	0.9888
0.05	16.6	130.9	0.9866
0.10	21.0	11.5	0.9995
0.50	25.8	-81.3	0.9973

Table 2. Effect of pH of phosphate buffer.

pH of phosphate buffer	Calibration data		
	Slope (mV.L/mg)	Y – intercept (mV)	r^2
3.0	24.7	-32.9	0.9976
5.6*	21.9	-48.5	0.9968
7.3	23.0	-41.6	0.9964
11.1	18.4	-81.3	0.9937

*Not adjusting pH

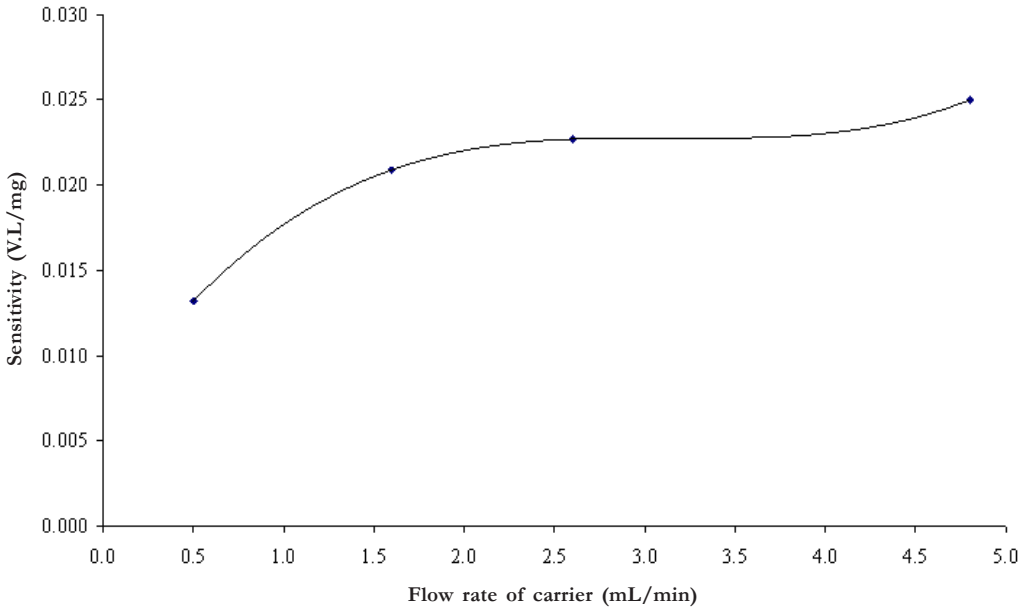

Figure 3. Effect of flow rate of carrier stream on sensitivity of ascorbic acid determination.

obtained. The flow rate of 1.5 mL/min was chosen because it provided enough sensitivity and sample throughput (20 injections per hour) with lower reagent consumption.

3.2 FI Amperometric System with Dialysis Unit

Dialysis unit was incorporated to the FI system as shown in Figure 1, in order to separate ascorbic acid from other matrices in samples. A cellulose acetate membrane of molecular weight cut off of 12,000 Da was utilized as a dialysis membrane. Employing the selected conditions obtained in section 3.1, ascorbic acid solutions in concentration range of 50 – 500 mg/L were injected. A linear calibration graph ($y=6x10^{-5}x$ -0.0006, r^2 = 0.9915) was obtained. Due to low dialysis efficiency (about 0.3%), a decrease in sensitivity was observed. However, it is still useful for determination of ascorbic acid in pharmaceutical and fruit juice samples, which high concentration of ascorbic acid is concerned. By on-line dialysis, dilution factor of about 300 (calculated from the ratio of the slope of calibration graphs from the systems without and with dialysis unit) could be reproducibly achieved.

3.3 Analytical Characteristics

Using the recommended conditions as described in section 2.2, a linear calibration graph in the range of 50 – 800 mg/L ascorbic acid was obtained ($y = 5x10^{-5}x$-0.0023 , r^2 = 0.9919). Detection limit calculated from the calibration data[26] was found to be 45 mg/L. The relative standard deviation of 10 replicate injections was 1.5 % for 50 mg/L of ascorbic acid, indicated a good precision of the method. Each injection consumed about 5 mL of reagent and 150 μL of sample.

3.4 Application of the Developed Method to Real Samples

3.4.1 Vitamin C Tablet Samples

The vitamin C samples from local drugstores were analyzed for ascorbic acid by the proposed method. All samples were

Table 3. Ascorbic acid contents in vitamin C tablet samples.

Sample	Label (mg/tablet)	Ascorbic acid found (mg/tablet)				% Different[c]
		FI – amperometric method [a]	% label	Voltammetric method [b]	%label	
1. Bio C 1000	1000	1021±6	102	1143±23	114	-11
2. Nat C	1000	998±15	100	1018±15	102	-2.0
3. Berocca	500	487±3	97	539±24	107	-9.6
4. C-500 nopparat	500	491±13	98	588±24	117	-16
5. Mag - C 500	500	525±8	105	615±15	123	-15
6. Flavettes	250	245±23	98	316±13	126	-22
7. Vitacimin	100	97±1	97	117±2	117	-17
8. Vit C 50 Frx	50	49±1	98	58±1	116	-16

[a] triplicate results
[b] duplicate results
[c] % different = [(FI value – Voltammetric value) x100] / Voltammetric value

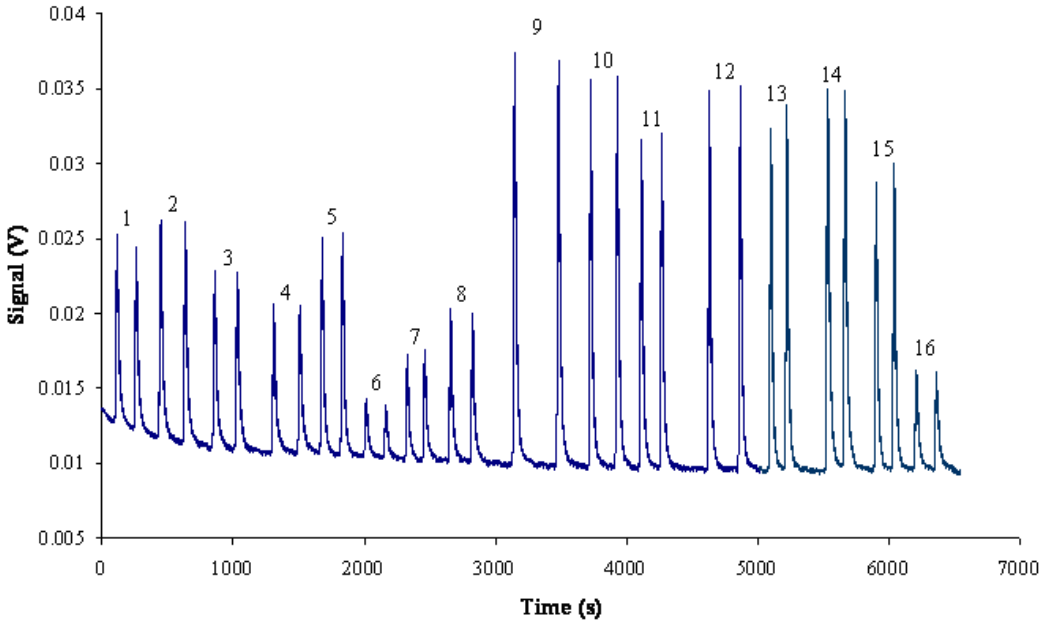

Figure 4. FIgram obtained from injecting of various fruit juice samples.

Table 4. Ascorbic acid contents in fruit juice samples

Sample No.	Type of sample	Label (mg/L)	Ascorbic acid found (mg/L)	
			FI – amperometric method	%label
1	Orange juice	-	292±6	-
2	Orange juice	-	337±4	-
3	Orange juice	480	280±2	58
4	Guava juice	-	244±1	-
5	Apple juice	-	337±7	-
6	Orange mixed carrot juice	-	118±4	-
7	Blueberry mixed apple juice	180	188±2	104
8	Orange mixed banana juice	180	247±3	137
9	Tangerine juice	330	587±4	178
10	Sai Nam Phueng orange juice	-	563±4	-
11	Shogun orange juice	420	488±3	116
12	Guava juice	360	554±4	153
13	Kiwi and grape juice	480	515±18	107
14	Pineapple juice	-	549±4	-
15	Broccoli and fruit juice	-	440±16	-
16	Mixed vegetable and mixed fruit juice	-	175±1	-

prepared as described in section 2.1. Concentration of ascorbic acid in the sample solution was calculated from peak height obtained using the calibration graph. Accuracy of the proposed method was determined by comparing results from FI-amperometric method with those obtained from voltammetric method [25] and the labeled values. The voltammetric method was used because it does not interfere by the intense color of sample. The results are summarized in Table 3. It was found that the results obtained from voltammetric method had a more positive bias than those obtained by FI-amperometric method. Regression equation: Ascorbic acid content by voltammetric method = 1.05 (Ascorbic acid content by FI method) + 34.6; r^2 = 0.9909, indicated a good correlation between the two methods (the slope is about 1.0). The positive bias obtained from voltammetric method may be due to interference of other ingredients such as multivitamins. This problem is avoided by using FI-amperometric system with dialysis sample pretreatment. According to t-test[26] at 95% confidence level, the results obtained from the proposed method agreed well with the labeled values. Moreover, the

developed method consumed small amounts of reagent and sample per analysis, and is faster than voltammetric method.

3.4.2 Fruit Juice Samples

Preliminary investigation on application of the developed system to the determination of ascorbic acid in fruit juice samples was carried out. Each sample was injected in duplicate and FIgram was obtained as shown in Figure 4. Ascorbic acid contents determined by FI-amperometric method and the labeled one are summarized in Table 4. In most of the samples, ascorbic acid concentrations found are higher than the labeled values. More investigations including comparison of the developed method with other methods (e.g., voltammetric and chromatographic methods) will be performed. It should be noted that the proposed method should be more appropriate than spectrophotometric and titrimetric ones for fruit juice analysis since the amperometric detection does not suffer from colored and colloidal interferences and Schileren's effect.

4. CONCLUSION

A new FI amperometric system with on-line dialysis sample pretreatment has been developed. The proposed system has many advantages such as low chemical consumption, high sample throughput, no interference from colored substances and colloids, good sensitivity and precision, and a simple bare GCE could be employed. Application to pharmaceutical and fruit juice samples was demonstrated. The method may be useful for research and quality control of food and pharmaceutical products which a large number of samples is involved.

ACKNOWLEDGEMENTS

We would like to acknowledge the Center for Innovation in Chemistry: Postgraduate Education and Research Program in Chemistry (PERCH-CIC), the Thailand Research Fund (TRF) and the Commission on Higher Education (CHE) for financial support. Thanks to Dr. Ponlayuth Sooksamiti, Dr. Supaporn Kradtap Hartwell and Dr. Kate Grudpan for useful discussions.

REFERENCES

[1] McCormick D.B., in: N.W. Tietz(Ed.), *Textbook of Clinical Chemistry,* Sanders, Philadelphia, PA, 1986.

[2] Davies M.B., Austin J. and Partridge, S.A., *Vitamin C: Its Chemistry and Biochemistry,* The Royal Society of Chemistry, Cambridge, 1991.

[3] Janghel E.K., Gupta V.K., Rai M.K. and Rai J.K., Micro determination of ascorbic acid using methyl viologen, *Talanta*, 2007; ***72***: 1013-1016.

[4] Sata Y., Kato D., Niwa O. and Mizutani F., Simultaneous determination of glucose and ascorbic acid by using gold electrode modified with ferrocenylundecanethiol monolayer, *Sens. Actuators B,* 2005; **108**: 617-621.

[5] Matos R.C., Augelli M.A., Pedrotti J.J., Lago C.L. and Angnes L., Amperometric Differential Determination of Ascorbic Acid in Beverages and Vitamin C Tablets Using a Flow Cell Containing an Array of Gold Micro-electrodes Modified with Palladium, *Electroanal.*, 1998; **10**: 887.

[6] Fontannaz P., Takiline A. and Heudi O., HPLC-UV determination of total vitamin C in a wide range of fortified food products, *Food Chem.*, 2006; **94**: 626-631.

[7] Wu T., Guan Y. and Ye J., Determination of flavonoids and ascorbic acid in grapefruit peel and juice by capillary electrophoresis with electrochemical detection, *Food Chem.*, 2007; **100**: 1573-1579.

[8] John S.A., Simultaneous determination of uric acid and ascorbic acid using glassy carbon electrodes in acetate buffer solution*, J. Electroanal. Chem.*, 2005; **579**: 249-256.

[9] Tian L., Chen L., Liu L., Lu N., Song W. and Wu H., Electrochemical determination of ascorbic acid in fruits on a vanadium oxide polypropylene carbonate modified electrode, *Sens. Actuators B,* 2006; **113**: 150-155.

[10] Yebra-Biurrun M.C., Flow injection determination methods of ascorbic acid, *Talanta*, 2000; **52**: 367-383.

[11] Lazaro F., Rýos A., Luque de Castro M.D., and Valcarcel M., Determination of vitamin C by flow injection analysis, *Analyst*, 1986; **111**: 163-166.

[12] Koupparis M.A., Anagnostopoulou P. and Malmstadt H.V., Automated flow-injection pseudotitration of strong and weak acids, ascorbic acid and calcium, and catalytic pseudotitrations of aminopolycarboxylic acids by use of a microcomputer-controlled analyzer, *Talanta*, 1985; **32**: 411-417.

[13] Sultan S.M., Abdennabi A.M. and Suliman F.E.O., Flow injection colorimetric method for the assay of vitamin C in drug formulations using tris,1-10-phenanthroline-iron(III) complex as an oxidant in sulfuric acid media, *Talanta*, 1994; **41**: 125-130.

[14] Pereira A.V. and Fatibello-Filho O., Spectrophotometric flow injection determination of L-ascorbic acid with a packed reactor containing ferric hydroxide, *Talanta*, 1998; **47**: 11-18.

[15] Pereira A.V. and Fatibello-Filho O., Flow injection spectrophotometric determination of L-ascorbic acid in pharmaceutical formulations with on-line solid-phase reactor containing copper (II) phosphate, *Anal. Chim. Acta*, 1998; **366**: 55-62.

[16] Sultan S.M., Flow injection titrimetric analysis of vitamin C in pharmaceutical products, *Talanta*, 1993; **40**: 593-598.

[17] Abdalla M.A. and Al-Swaidan H.M., Iodimetric determination of iodate, bromate, hypochlorite, ascorbic acid and thiourea using flow injection amperometry, *Analyst*, 1989; **114**: 583-586.

[18] Albero M.I., Garcýa, M.S., Sanchez-Pedreno, C., and Rodrýguez, J., Determination of ascorbic acid in pharmaceuticals and urine by reverse flow injection, *Analyst*, 1992; **117**: 1635-1638.

[19] Leon, L.E., Amperometric flow-injection method for the assay of l-ascorbic acid based on the photochemical reduction of Methylene Blue, *Talanta*, 1996; **43**: 1275-1279.

[20] Grudpan, K., Kamfoo, K., and Jakmunee, J., Flow injection spectrophotometric or conductometric determination of ascorbic acid in a vitamin C tablet using permanganate or ammonia, *Talanta*, 1999; **49**: 1023-1026.

[21] Messina, G.A., Torriero, A.A.J., De Vito, I.E., and Raba, J., Continuous-flow/stopped-flow system for determination of ascorbic acid using an enzymatic rotating bioreactor, *Talanta*, 2004; **64**: 1009-1017.

[22] Paixao, T.R.L.C., and Bertotti, M., FIA determination of ascorbic acid at low potential using a ruthenium oxide hexacyanoferrate modified carbon electrode, *J. Phar. Biomed. Anal.*, 2008; **46**: 528-533

[23] Grudpan, K. , Sritharathikhun, P., and Jakmunee, J., Flow injection conductimetric or spectrophotometric analysis for acidity in fruit juice, *Anal. Chim. Acta*, 1998; **363**: 199-202.

[24] Tue-Ngeun, O., Jakmunee, J., and Grudpan, K., A novel stopped flow injection—amperometric procedure for the determination of chlorate, *Talanta*, 2005; **68**: 459-464

[25] Poouthree, K., *MS Thesis in Chemistry,* Chiang Mai University, 2000.

[26] Miller, J.C. and Miller, J.C., *Statistics for Analytical Chemistry,* 3rd ed., Ellis Horwood, West Sussex, 1993.