CHAPTER 3

RESULTS AND DISCUSION

3.1 Determination of fumaric acid

A differential pulse voltammetric (DPV) system was developed for the determination of fumaric acid. It involves the electroreduction of fumaric acid on a hanging mercury drop electrode (HMDE). A peak current at about -1150 mV was obtained when scanning potential in the range of -900 to -1300 mV versus Ag/AgCl electrode. Effects of electrolyte solution and voltammetric parameters on the determination were investigated. Development of simple devices for FI voltammetric system and attempt to applying the system for determination of fumaric acid was carried out.

3.1.1 Batchwise DPV system

A batchwise differential pulse polarographic (DPP) analysis is the standard method of the Association of Official Analytical Chemists (AOAC) for determination of fumaric acid [6]. Details of the method could be seen in appendix. This method used dropping mercury electrode (DME), which high amounts of toxic mercury waste are generated. Attempt to reduce mercury waste by adapting the standard method to be DPV method, which used only 1 drop of mercury (HMDE) per analysis was made [65]. In this research, effects chemical parameters (type, concentration and pH of supporting electrolyte) and voltammetric parameters (potential amplitude (U.amp),

potential step (U.step), measuring time (t.meas), step time (t.step) and pulse time (t.pulse)) on DPV determination of fumaric acid were thoroughly investigated.

3.1.1.1 Preliminary investigation on DPV system

The DPV method adapting from AOAC standard method[6] was preliminary investigated for determination of fumaric acid. The instrument setup as described in section 2.3.1 was employed. A fumaric acid standard solution (100 mg/L) was successively spiked (0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mL) into 10 ml of supporting electrolyte (0.1M $(CH_3)_4NBr$ + 0.01M LiCl) placing in a voltammetric cell. The solution was purged with nitrogen gas for 3 min to remove dissolved oxygen before starting the DPV analysis by using the condition as shown in Table 3.1.

Table 3.1 Condition for batchwise DPV analysis

Parameter	**Condition**
Electrode: 1.Working electrode 2. Reference electrode 3. Auxiliary electrode	 Hanging mercury drop electrode Ag/AgCl Pt wire
Potential amplitude (U.ampl)	50 ms
Measurement time (t. meas)	20ms
Step time (t. step)	0.30 s
Pulse time (t. pulse)	40.0 ms
Potential step (U. step)	10 mV
Scanning potential: Initial potential Final potential	 -600 mV -1300 mV

Under the preliminary condition, it was found that the peak current of fumaric acid occurred at –1050 mV versus Ag/AgCl as summarized in Table 3.2 and a linear

calibration graph in range of 1-10 mg/L fumaric acid was obtained as shown in Figure 3.1.

Table 3.2 Peak current obtained for 1.0 – 9.9 mg/L fumaric acid

Concentration of fumaric acid (mg/L)	Peak current (nA)[a]
1	2
2	30
3.8	76
5.7	125
7.4	172
9.1	204

[a] = mean of triplicate results

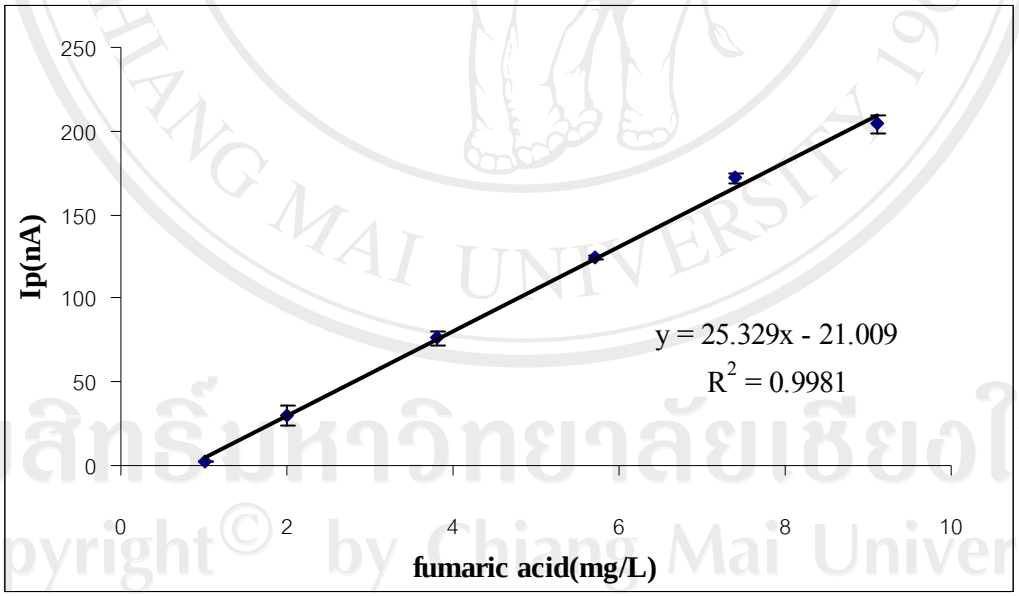


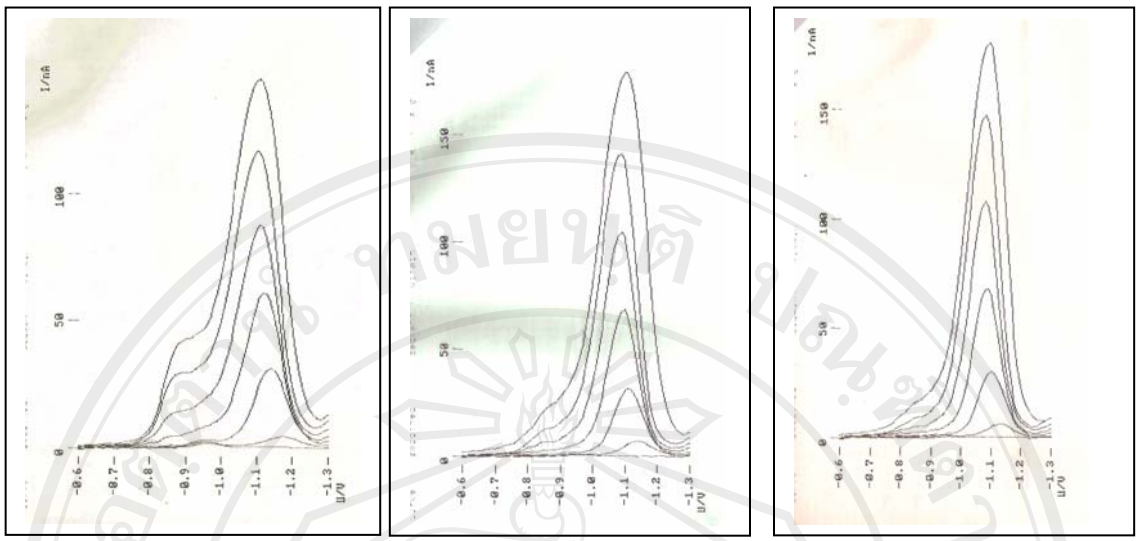
Figure 3.1 A calibration graph for batchwise DPV determination of fumaric acid by successive spiking standard solution of fumaric acid into an electrolyte.

3.1.1.2 Effect of tetramethylammonium bromide concentration

Effect of tetramethylammonium bromide concentration was studied by varying its concentration in range of 0.01 – 0.50 M, while lithium chloride concentration was fixed at 0.01 M. Voltammogram of standard fumaric acid solution (1.0-9.1 mg/L) was measured as illustrated in Figure 3.2. Calibration graphs were constructed by plotting peak current versus fumaric acid concentration. Slopes, intercepts and r^2 of the calibration graphs are shown in Table 3.3.

Table 3.3 Calibration graph data using various tetramethylammonium bromide concentrations.

Concentration of tetramethylammonium bromide (M)	Calibration graph data		
	Slope (nA/mgL^{-1})	Y – intercept (nA)	r^2
0.01	11.5	9.7	0.9950
0.05	16.8	-2.3	0.9919
0.1	18.7	-7.6	0.9923
0.2	19.9	-10.9	0.9948
0.5	20.2	-13.4	0.9952



0.01 M $(CH_3)_4NBr$ 0.05 M $(CH_3)_4NBr$ 0.1 M $(CH_3)_4NBr$

Figure 3.2 Example of voltammogram signal profiles obtained for fumaric acid determination (1, 2, 3.8, 5.7, 7.4 and 9.1 mg/L).

It was found that at the higher concentration of tetramethylammonuim bromide the higher sensitivity was obtained. The 0.1M tetramethylammonuim bromide was chosen because it gives nearly the same sensitivity as 0.2 and 0.5 M, but smaller amounts of chemical was employed.

3.1.1.3 Effect of lithium chloride concentration

The lithium chloride concentration was varied from 0.1 x 10^{-2} – 3.0 x 10^{-2} M, while tetramethylammonuim bromide concentration was fixed at 0.1 M. Voltammograms of standard fumaric acid (1.0-9.1 mg/L) were measured and the Slopes, intercepts and r^2 of the calibration graphs are summarized in Table 3.4.

Table 3.4 Calibration graph data using various lithium chloride concentrations.

Concentration of lithium chloride x 10^{-2} (M)	Calibration graph data		
	Slope (nA/mgL^{-1})	Y – intercept (nA)	r^2
0.1	18.3	-4.8	0.9901
0.5	18.4	-5.5	0.9908
1.0	18.1	-4.0	0.9914
2.0	16.4	5.1	0.9677
3.0	18.3	-3.2	0.9855

It was found that all concentrations of lithium chloride gave closely sensitivity but at the lower concentration than 1.0x10^{-2}M it provided better r^2. However, 1.0 x 10^{-2}M lithium chloride was chosen because it gave a better background signal than 0.1 x 10^{-2}M and 0.5 x 10^{-2}M will be interfere for measuring very low concentration of fumaric acid.

3.1.1.4 Effect of sodium chloride concentration

Sodium chloride was investigated to be used as a cost effective alternative reagent in stead of lithium chloride. Its concentrations in range of 0.01-0.06 M were studied. It was found that all concentrations of sodium chloride gave closely sensitivity. However, 0.02 M sodium chloride was chosen because it provided better r^2.

3.1.1.5 Effect of pH of electrolyte solution

The pH of electrolyte solution should affect the electroreduction of fumaric acid at a working electrode because hydrogen ion involves in the reaction. Supporting electrolyte of various pH values were prepared by adjusting the pH with

sodium hydroxide or hydrochloric acid. Voltammograms of standard fumaric acid solutions (1.0-9.1 mg/L) was measured and the slopes, intercepts and r^2 of the calibration graphs are summarized in Table 3.5.

Table 3.5 Calibration graph data using various supporting electrolyte pH values.

Supporting electrolyte pH values	Calibration data		
	Slope (nA/mgL^{-1})	Y – intercept (nA)	r^2
2.6	16.7	0.4	0.9824
3.2	13.9	-38.8	0.8729
3.9	19.7	-17.5	0.9821
5.6	20.2	-9.5	0.9983
8.1*	20.1	-10.3	0.9967
10.8	0.6	-1.3	0..6066

*not adjusting pH value.

The pH of supporting electrolyte at 5.6 and 8.1 gave closely sensitivity and higher than other pH values. However buffer of pH 8.1 was chosen because this solution is easier to prepare. In basic solution (pH 10.8), very small peak current was observed.

3.1.1.6 Effect of voltammetric parameters

Voltammetric parameters should be studied to improve signal to noise ratio of fumaric acid determination. The potential waveform of DPV is illustrated in Figure 3.3 with the details of the involved parameters. Voltammograms of standard fumaric acid solutions in concentration range of 10 – 25 mg/L were recorded. The slopes, intercepts and r^2 of the calibration graphs obtained for each parameter studied are shown in Table 3.6-3.10.

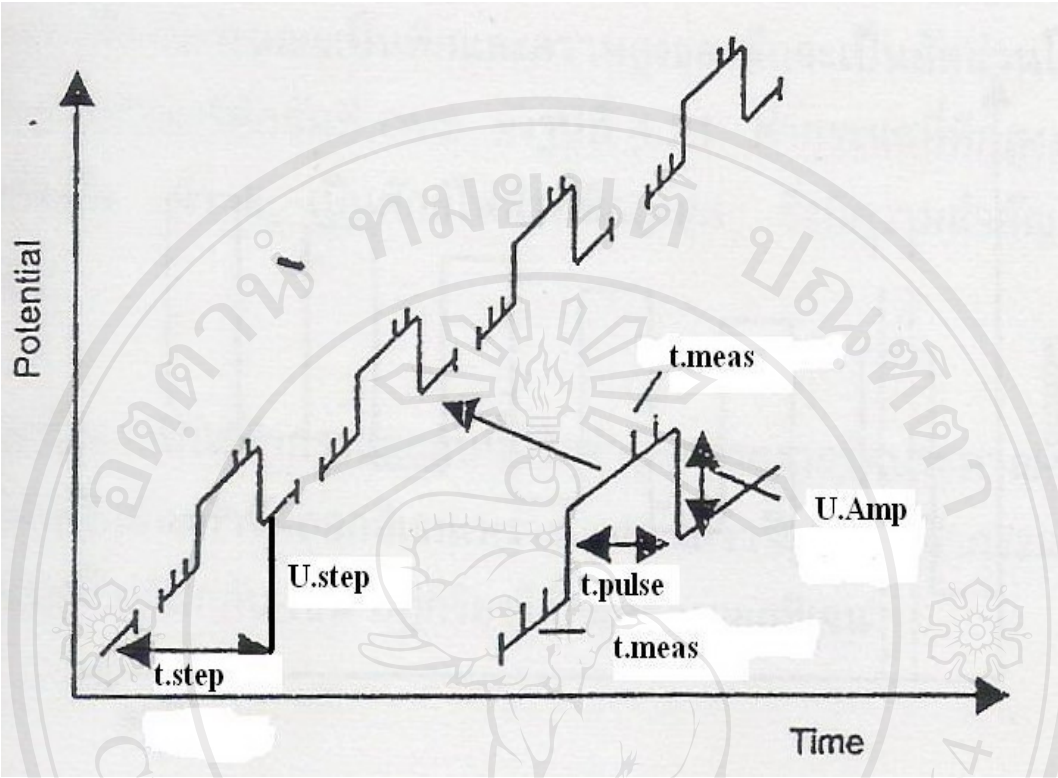


Figure 3.3 The Potential waveform for DPV.

Table 3.6 Calibration graph data using various potential pulse amplitudes (U.amp)

U.amp (mV)	Calibration graph data		
	Slope (nA/mgL^{-1})	Y – intercept (nA)	r^2
5	1.7	-13.4	0.9928
25	5.8	-43.2	0.9853
50	9.6	-81.5	0.9943
75	11.7	-103.4	0.9834
100	14.0	-132.1	0.9960

Table 3.7 Calibration graph data using various potential steps (U.step).

U.step (mV)	Calibration graph data		
	Slope (nA/mgL^{-1})	Y – intercept (nA)	r^2
4	7.3	-58.8	0.9888
8	7.8	-57.1	0.9994
10	9.0	-63.1	0.9912
12	9.6	-81.5	0.9943

Table 3.8 Calibration graph data using various measuring times (t.meas)

t.meas(ms)	Calibration graph data		
	Slope (nA/mgL^{-1})	Y – intercept (nA)	r^2
1	9.9	-77.1	0.9969
20	11.3	-102.2	0.9911
30	11.4	-104.7	0.9992

Table 3.9 Calibration graph data using various step times (t.step)

t.step (s)	Calibration graph data		
	Slope (nA/mgL^{-1})	Y – intercept (nA)	r^2
0.05	23.8	-241.8	0.9961
0.1	20.7	-184.7	0.9995
0.2	17.5	-152.2	0.9943
0.25	10.7	-98.0	0.9961
0.6	7.1	-65.0	0.9968
1.2	5.7	-53.8	0.9961

Table 3.10 Calibration graph data using various pulse time (t.pulse)

t.pulse (ms)	Calibration graph data		
	Slope (nA/mgL^{-1})	Y – intercept (nA)	r^2
40	18.8	-201.2	0.9889
60	20.7	-184.7	0.9995
68	14.9	-144.6	0.9977

3.1.1.7 Summary of the selected conditions

The selected concentration of reagents; tetramethylammonium bromide and lithium chloride for determination of fumaric acid in the range of 1 – 10 mg/L are 0.1 and 0.01 M, respectively. The pH of supporting electrolyte of about 8 (without adjustment of pH) was selected. The studied ranges and the selected values of various voltammetric parameters are summarized in Table 3.11.

Table 3.11 Studied range and the selected values of various voltammetric parameters

Parameter	Variable value	Selected value for FI-DPV
Potential amplitude (U.amp), mV	5-100	100
Potential step (U.step), mV	4-12	12
Measurement time (t.meas), ms	1-30	20
Step time (t.step), s	0.05-1.2	0.1
Pulse time (t.pulse), ms	40-68	60

From Table 3.6, the maximum potential pulse amplitude (U.amp) at 100 mV was chosen because it provided higher peak current than another potential pulse amplitudes. Likewise, potential step (U.step) was chosen, increasing potential step provide potential sweep rate that be suitable for flow system.

According to the relations of timing parameters step time (t.step > t.pulse + t.meas + 30ms), pulse time (t.pulse > t.meas + 2ms) and measurement time (t.meas range 1.0-32.0ms) for voltammograph model VA 693, the suitable parameters were chosen to provide better sensitivity and avoid the limit of instrument.

3.1.2 Investigation on FI DPV system for determination of fumaric acid

FI procedure usually provides rapid analysis, low reagent consumption and high degrees of automation. In this section, a simple FI DPV system for determination of fumaric acid was tried.

3.1.2.1 Development of cost effective alternative propelling devices

A peristaltic pump is usually used in the FI system because it offers precise flow with easy control of flow rate. However, the movement of the peristaltic pump rollers causes pulsation flow which interferes in the voltammetric measurements. The peristaltic pump is also relatively expensive. Some alternative propelling devices were investigated in this research, i.e., gas pressure and gravity driven pumping devices.

Nitrogen gas pressure driven pumping system

Nitrogen gas pressure driven pumping system is interesting to be used as a propulsion system in FI DPV system because it should offer an in situ oxygen removal from the electrolyte solution. The system was constructed as described in section 2.3.2.1. With constant N_2 pressure at 1.5 bars applied on top of a liquid in a closed bottle, constant flow rate can be achieved by adjusting a restriction valve on liquid side.

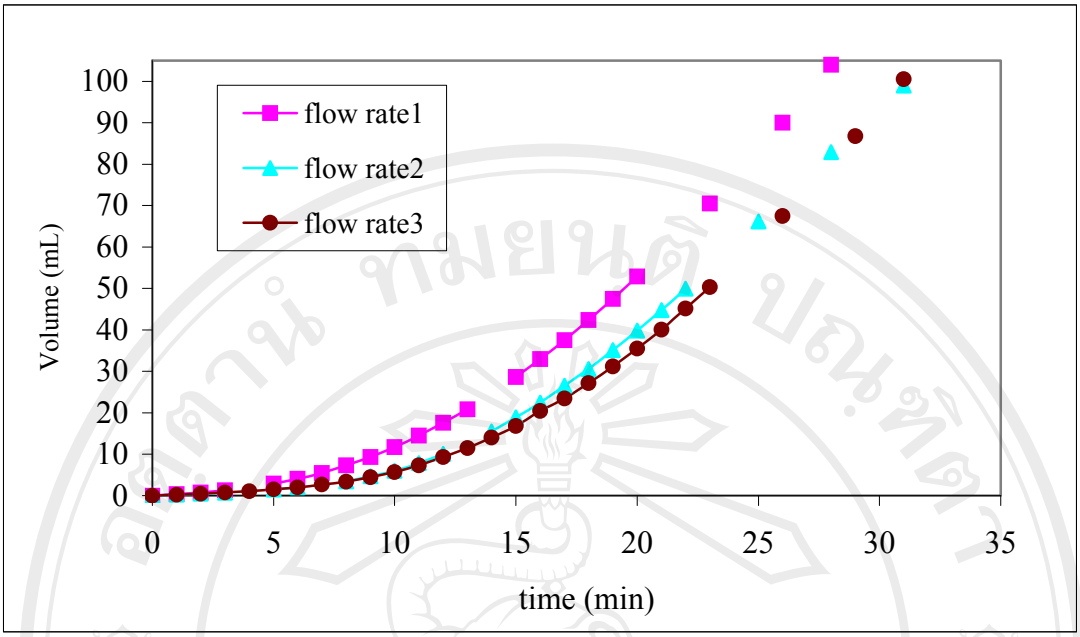


Figure 3.4 Different flow rates achieved from N_2 gas pressure driven system.

As shown in Figure 3.4, a constant flow rate (constant slopes) with pulse – free flow could be obtained at least 15 min after starting the flow. This may due to the flow rate depends on level of liquid in a bottle. This pumping system is not investigated further because it needs too long start up time and it occasionally generated bubbles in the line.

Gravity driven pumping system

A gravity driven pumping system based on the mariotte bottle was studied to be used as propelling device in FIA system. The device is described in section 2.3.2.2. By adjusting a restriction valve, a constant liquid flow rate could be controlled. Performance of this pumping device is demonstrated in Figure 3.5.

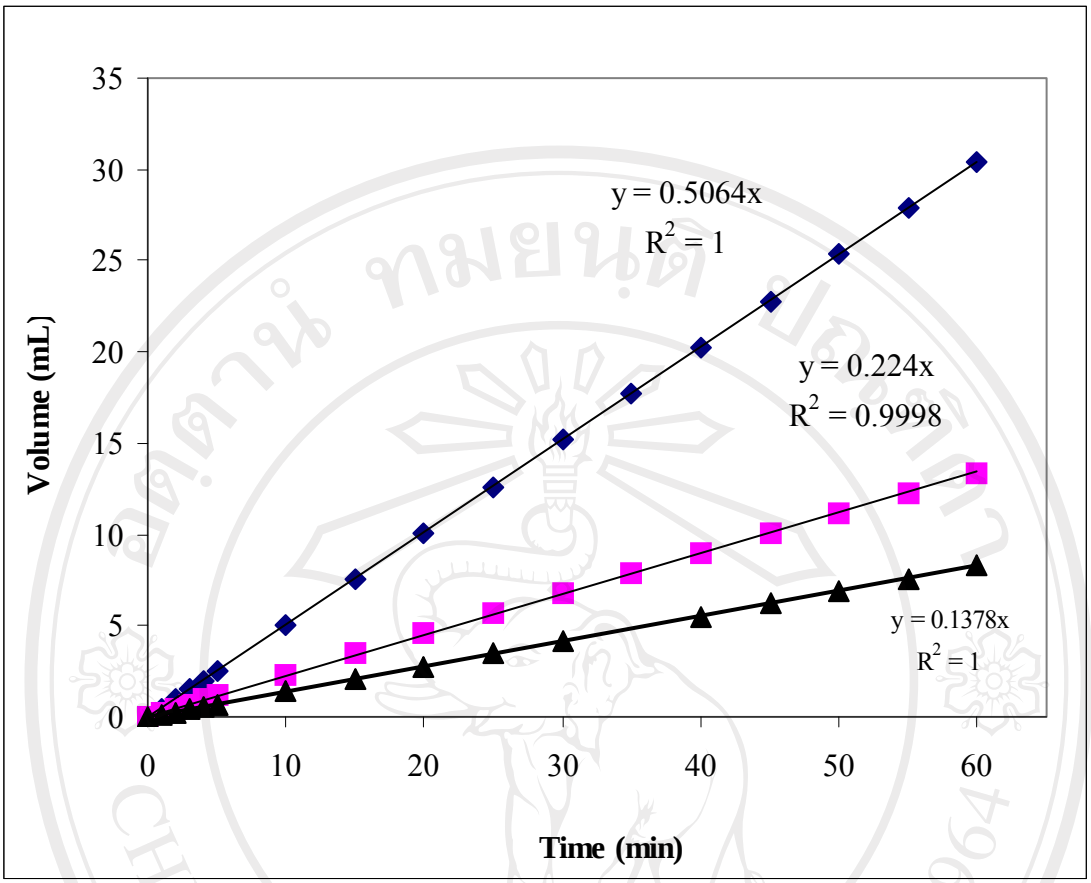


Figure 3.5 Different flow rates achieved from gravity driven pumping system: constant flow rates of 0.13, 0.22 and 0.51 mL/min were obtained.

Using this device, a constant flow rate with pulse – free flow was obtained with no need of the start up time, so it was selected for further experiments.

3.1.2.2 Development of a simple voltammetric flow cell

A voltammetric flow – through cell was constructed by adapting from a commercial batch voltammetric cell (Metrohm), similar to that previously reported [65]. A J – shaped Teflon tube of 0.5 mm diameter was inserted through a wall of a pipet tip

that used as a small vessel at the end of capillary tip of HMDE (see Figure 3.6). A solution was flowed to the HMDE and then went out to the solution outside the pipet tip vessel.

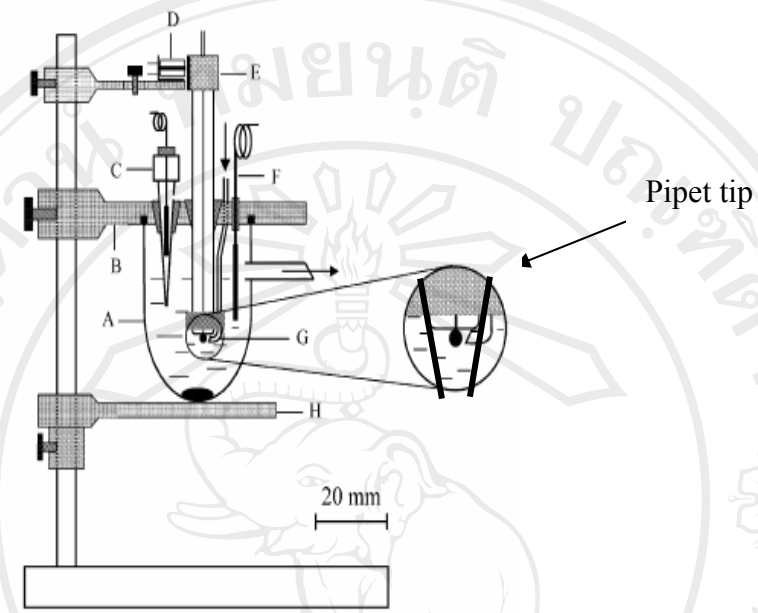


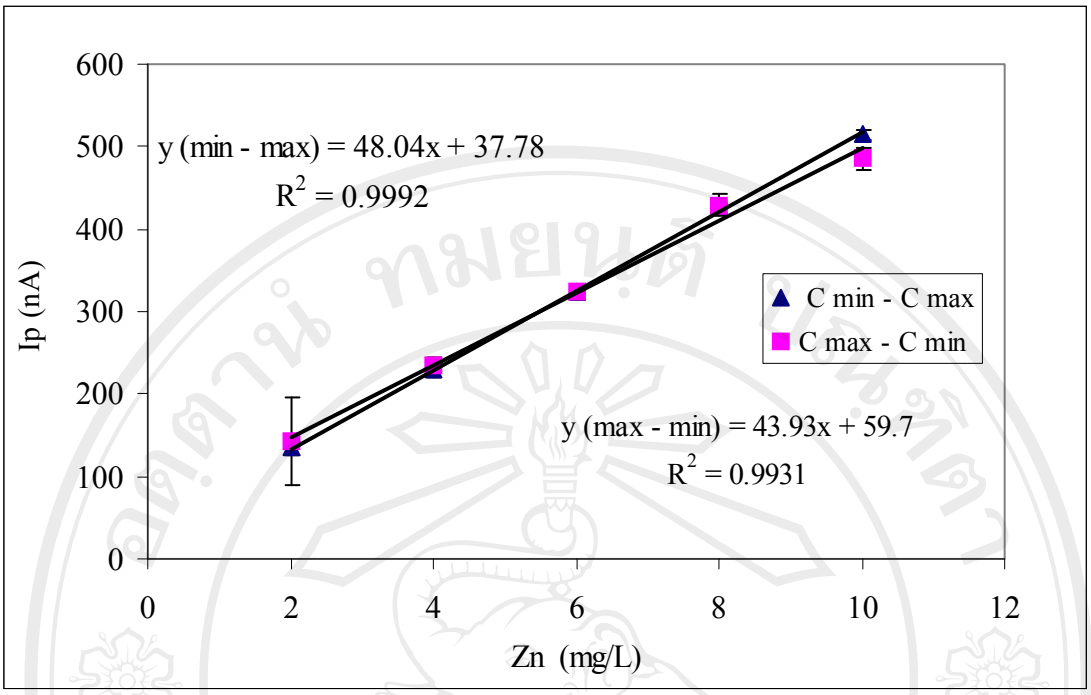
Figure 3.6 Cross section of voltammetric flow cell; (A)voltammetric vessel, (B)clamp, (C)Ag/AgCl reference electrode, (D)drop knocker, (E) HDME, (F)Pt electrode, (G) J-shaped adapter and (H) clamp. [adapted from ref. 66]

The developed flow cell was tested for FI DPV determination of zinc, in order to check the stability, reproducibility and carry over in the application of the electrode to FI. Zinc was chosen as a model analyte [67], because DPV of zinc on HMDE is well-known, has high sensitivity and zinc is not toxic as lead, cadmium or copper. The FI DPV system as described in section 2.3.3 was used with 0.1 M acetate buffer as a carrier and travelling time, time period for the injected zone to travel from injection point to the flow cell, of 90 s, measuring time of 30 s, and washing time of

10 s. By applying the DPV potential waveform as shown in Table 3.12 during the measuring time, a well-defined voltammogram was obtained. Linear calibration graphs were obtained in concentration range 2 - 10 mg/L Zn (see Figure 3.7) for either injecting standard solutions from low to high or high to low concentrations. The slopes of the calibration graphs are closely identical indicated that no carry over effect was involved.

Table 3.12 DPV parameters for zinc determination

Parameter	**Condition**
Electrode: 1.Working electrode 2. Reference electrode 3. Auxiliary electrode	 HMDE Ag/AgCl Pt wire
Potential amplitude (U.ampl)	50 ms
Measurement time (t. meas)	20ms
Step time (t. step)	0.30 s
Pulse time (t. pulse)	40.0 ms
Potential step (U. step)	10 mV
Scanning potential: Initial potential Final potential	 -800 mV -1200 mV
Flow rate	0.26 mL/min



Figures 3.7 Calibration graphs of Zn determination by injecting solution from minimum to maximum concentrations and maximum to minimum concentrations.

The same system was applied for FI – DPV for determination of fumaric acid, but by using a supporting electrolyte containing tetramethylammonium bromide (0.1M) and lithium chloride (0.01M) as a carrier and other conditions as summarized in Table 3.13. A series of working standard fumaric acid solution (0-80 mg/L) was injected. Voltammograms were obtained as shown in Figure 3.8.

Table 3.13 DPV parameters for fumaric acid determination.

Parameter	**Condition**
Electrode: 1.Working electrode 2. Reference electrode 3. Auxiliary electrode	 HMDE Ag/AgCl Pt wire
Potential amplitude (U.ampl)	100 ms
Measurement time (t. meas)	20ms
Step time (t. step)	0.10 s
Pulse time (t. pulse)	60 ms
Potential step (U. step)	10 mV
Scanning potential: Initial potential Final potential	 -600 mV -1600 mV
Flow rate	0.26 mL/min

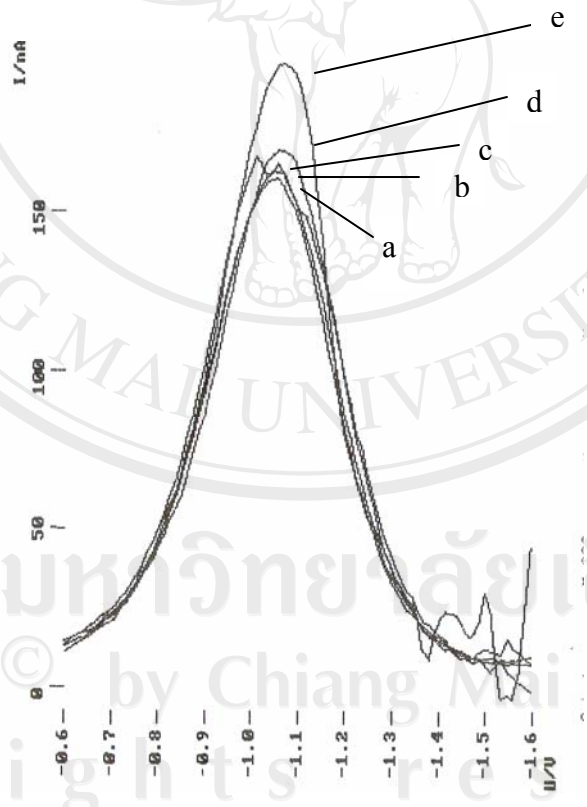


Figure 3.8 Voltammogram of fumaric acid at (a) 0(background), (b) 20, (c) 40, (d) 60 and (e) 80 mg/L.

From the voltammmograms, the high background signal was observed and peak current was not proportional to fumaric acid concentration. This may be due to the solution to flow directly to a mercury drop, so causing a high convective current. Thus, a new flow cell as shown in Figure 3.9 was designed to reduce convective current from convective mass transfer in flow injection system.

The previous flow cell was adapted by inserting the Teflon tube to the pipet tip at the angle which would direct the solution to flow to the inner wall of the pipet tip.

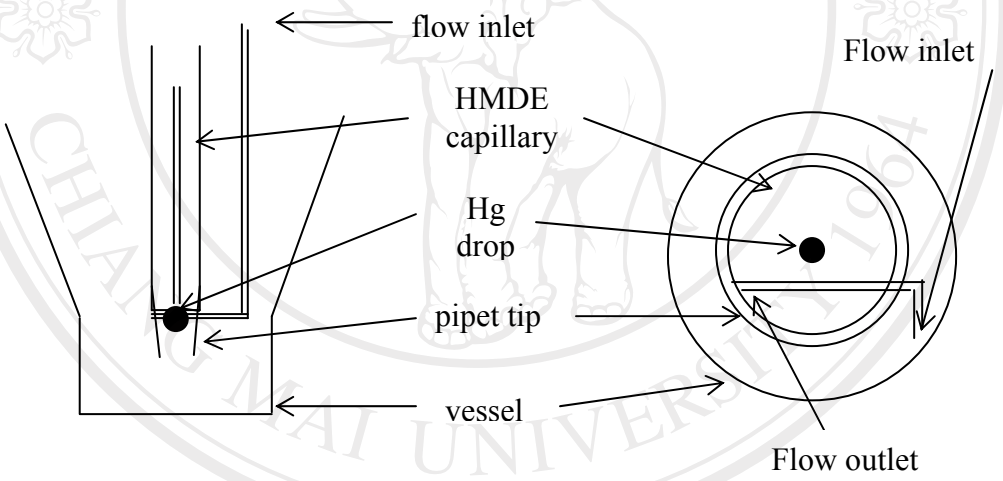


Figure 3.9 Side and bottom views of a new design of a laboratory made HMDE flow cell.

The new flow cell was tested by using supporting electrolyte as a carrier and voltammograms were scanned for background signals while the supporting electrolyte carrier solution was flowing. The analysis of 9 replicates was done under the selected condition as in Table 3.13. The results are shown in Table 3.14.

Table 3.14 Reproducibility of background current.

Number of measurement	I (nA)
1	78.48
2	77.58
3	75.15
4	74.55
5	72.12
6	78.08
7	72.67
8	75.08
9	74.17
Mean	75.32
SD	2.290
%RSD	3.040

From the results, it was found that the background current were lower than those obtain from the previous flow cell. The relative standard deviation (RSD) of background current obtained was 3.0%. This indicates that the proposed flow cell provides a good reproducibility.

3.1.2.3 FI - DPV determination of fumaric acid

Using the lab-built FI DPV system with conditions as in Table 3.13, a series of working standard (10, 20, 40, 60 and 80 mg/L) was injected. A calibration graph

(a plot of height of peak versus concentration of fumaric acid) is depicted in Figure 3.10 and the signal to noise ratio were obtained, as shown in Table 3.15.

Table 3.15 Peak height signal and signal to noise ratio of fumaric acid determination by FI – DPV.

Fumaric acid (mg/L)	Peak height (nA)	S/N
background	75.4	-
10	78.2	1.0
20	82.4	1.1
40	81.8	1.1
60	250.2	3.3
80	363.1	4.8

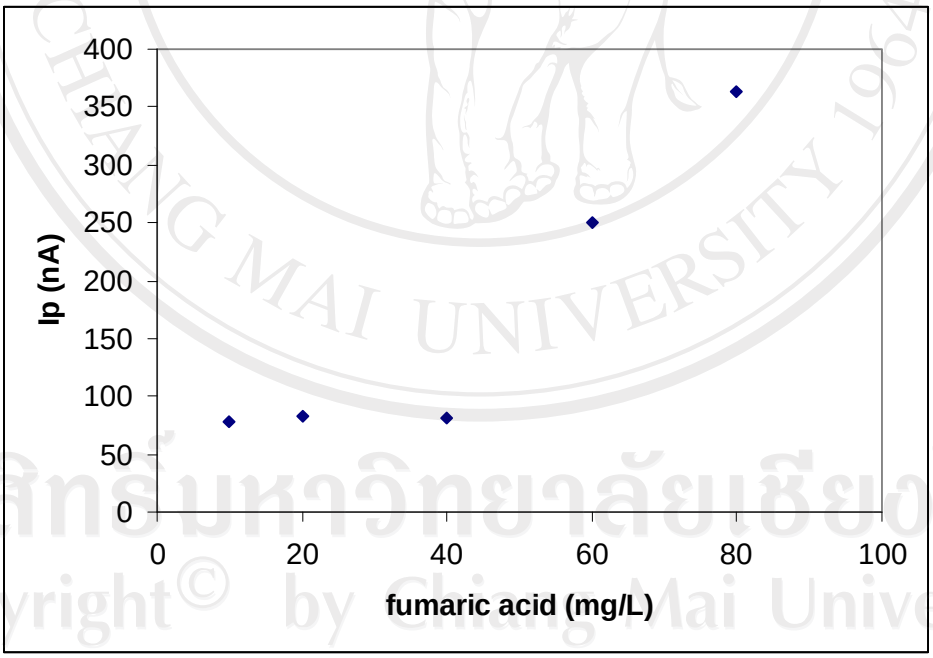


Figure 3.10 Calibration graph of fumaric acid by FI DPV.

From the results, although at high fumaric acid concentration a good signal to noise ratio was obtained, but a linear calibration graph could be achieved at concentration of fumaric acid higher than 40 mg/L, which is not applicable to fruit

juice analysis. The high background signal might be arise from convection in the flow cell while the potential was scanned. In order to prove the effect of flow rate, the background voltammetric signal was recorded at different flow rates, as the result is shown in Figure 3.11.

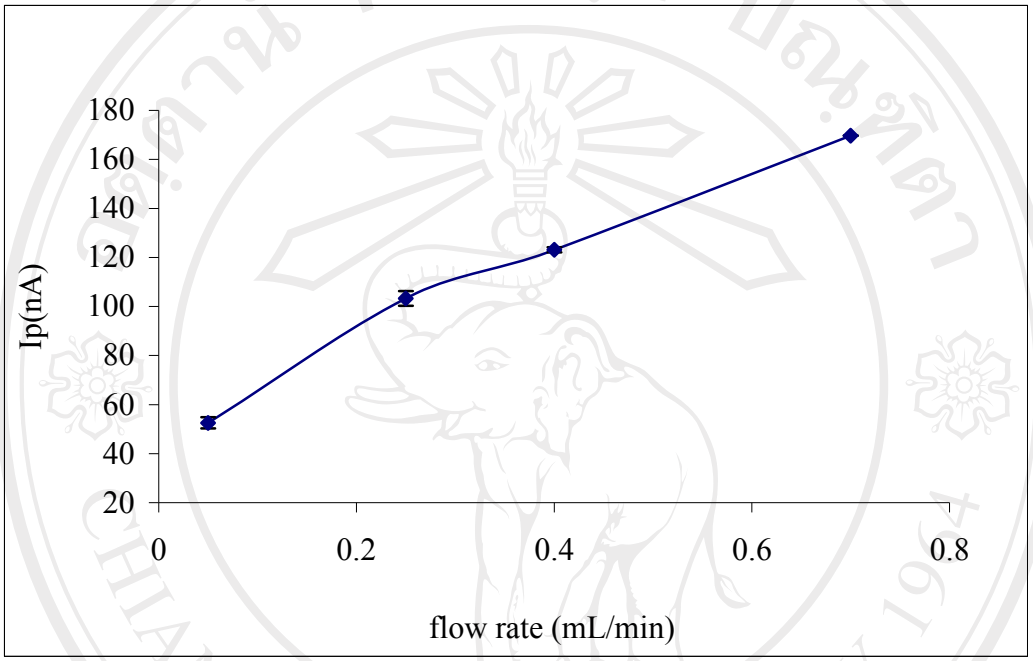


Figure 3.11 Relationship of background current with flow rate.

The background current is decreased when the mass transfer to HMDE is slow, i.e., using lower flow rate. However, it is still not enough to give good a signal to noise ratio to go to low fumaric acid concentration. Further experiments should be done, by using stopped FI while DPV scanning which may provide lower detection limit. Although the proposed procedure could not be used for determination of fumaric acid in fruit juice, this simple FI DPV may be useful for analysis of sample with high concentration of fumaric acid, e.g. solution from fermentation tank.

3.2 FI amperometric system for ascorbic acid determination

Most of the FI methods for assaying of ascorbic acid are based on spectrophotometric detection, employing the reducing property of ascorbic acid. However, colored or colloidal 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 (+0.4 to +0.6V vs Ag/AgCl) than the modified electrode, which may lead to electrode fouling, poor reproducibility and low selectivity, especially in batch procedure. Modified electrodes have been proposed to avoid these problems [37-38], but complicated process in preparation and limited stability of the electrode were found.

In this section, 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 is expected. The dialysis unit also provided convenient on-line dilution of sample.

3.2.1 Optimization of the FI Amperometric Detection System

A lab-built amperometer with a home-made data acquisition device was employed in the FI amperometric system as described in section 2.4.1. Preliminary conditions, 0.1 M phosphate buffer solution of pH 6.0 as a carrier solution, flow rate

1.0 mL/min, applied potential of 100 mV were employed. Effects of various parameters were investigated as followed.

3.2.1.1 Type of Working Electrode

Two types of working electrode, platinum disc (2 mm diameter) and glassy carbon disc (3 mm diameter) electrodes were investigated. A standard solution of 100 mg/L ascorbic acid was injected in 11 replicates into the system. The peak currents of 19.2±0.6 and 20.5±0.6 were obtained for Pt and GC electrodes, respectively, as shown in Table 3.16 and Figure 3.12. Despite both of the electrodes could be used, the GCE was selected for further experiment.

Table 3.16 Peak height of 100 mg/L ascorbic acid standard solution using platinum and glassy carbon electrodes as working electrode.

Electrode type	Average Signal (V)	SD	RSD
Pt electrode	19.24	0.64	3.3
Glassy carbon	20.54	0.58	2.8

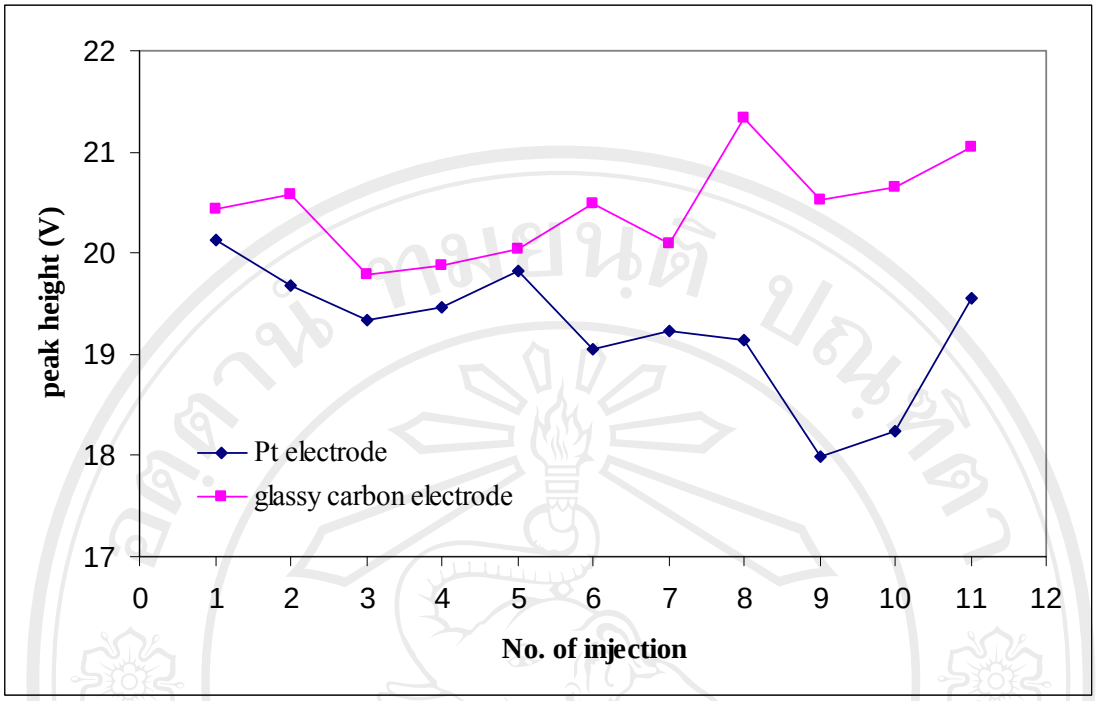


Figure 3.12 Comparison of peak heights of 100 mg/L ascorbic acid when using platinum and glassy carbon electrodes as working electrode.

3.2.1.2 Effect of applied potential

The potential applied on glassy carbon working electrode was varied in the range of 100 – 1400 mV vs. Ag/AgCl. A solution containing 40 mg/L of the working standard ascorbic acid was injected into the system to recorded peak height of each applied potential, as illustrated in Figure 3.13.

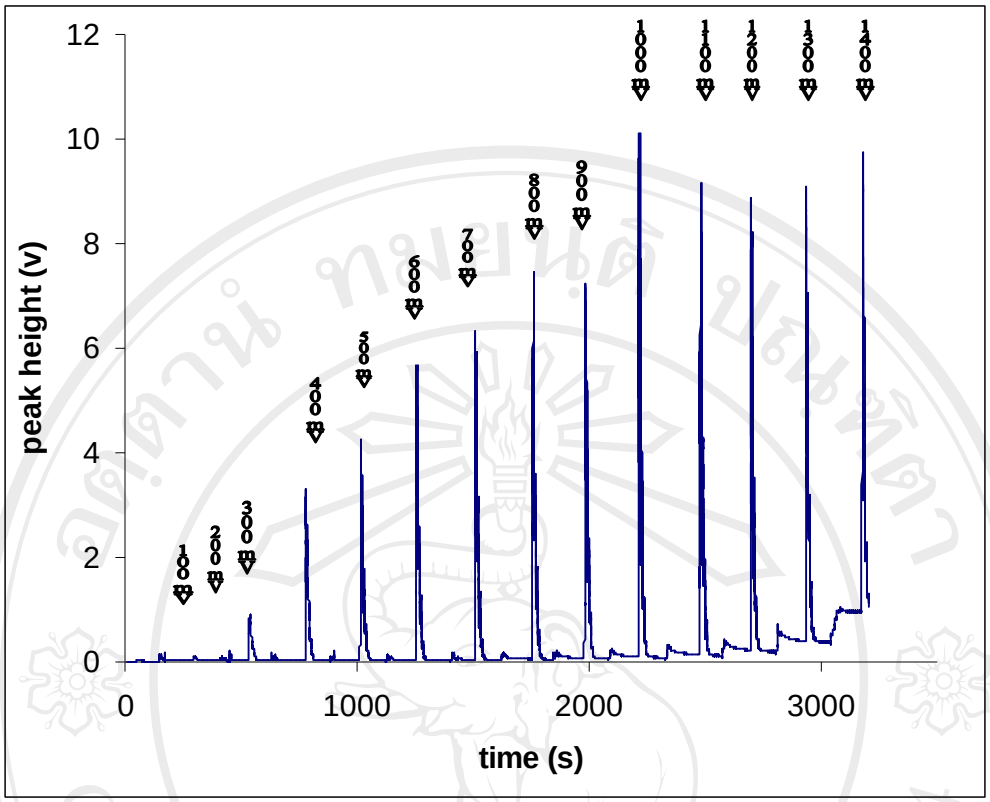


Figure 3.13 The peak height of ascorbic acid (40 mg/L) at each applied potential.

It was found that peak current obtained increased sharply with applied potential in range of 0.1-0.8 V and reached maximum at 1.0 V. 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.

3.2.1.3 Concentration and pH of Phosphate Buffer

Phosphate buffer solution was used as supporting electrolyte solution. The concentration of phosphate buffer was varied from 0.005 – 0.5 M while the pH of buffer was fixed at 6. FIgram of standard ascorbic acid solution was recorded. Calibration graphs in concentration range 20 – 100 mg/L ascorbic acid were constructed by plotting peak current vs. ascorbic acid concentration. Slopes,

intercepts and r^2 of the calibration graphs are shown in Table 3.17. Slope of the calibration graphs are plotted versus the concentration of phosphate buffer, as illustrated in Figure 3.14.

Table 3.17 Calibration graph data using different phosphate buffer concentration.

Concentration of phosphate buffer (M)	Calibration graph data		
	Slope (V/mgL^{-1})	Y – intercept (V)	r^2
0.005	0.0010	0.1649	0.9888
0.05	0.0166	0.1309	0.9866
0.1	0.0210	0.0115	0.9995
0.5	0.0258	-0.0813	0.9973

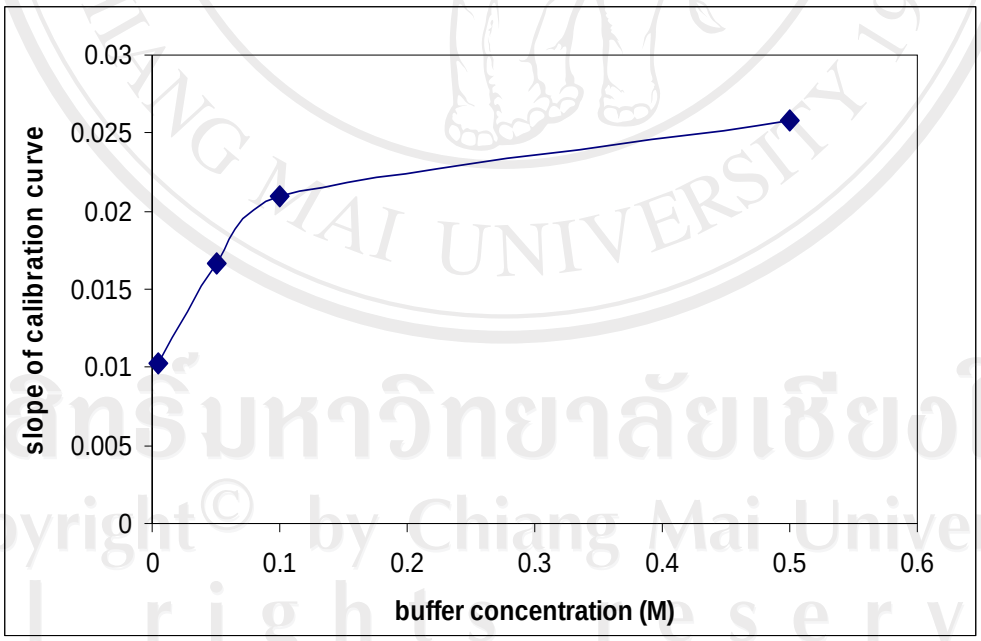


Figure 3.14 Effect of phosphate buffer concentration.

It was found that at concentration of phosphate buffer above 0.1 M, only slightly increased in sensitivity was obtained. Therefore, phosphate buffer concentration of 0.1 M was chosen because it provided high sensitivity with lower reagent consumption.

The pH of phosphate buffer was varied from 3 -11, while phosphate buffer concentration was fixed at 0.1 M. FIgram of various standard ascorbic acid solutions was recorded. Calibration graphs in concentration range of 20 – 100 mg/L were constructed. Slopes, intercepts and r^2 of the calibration graphs are shown in Table 3.18. Slope of the calibration graphs are plotted versus the pH of phosphate buffer, as illustrated in Figure 3.15.

Table 3.18 Calibration graph data for each phosphate buffer pH.

phosphate buffer pH	Calibration graph data		
	Slope (V/mgL^{-1})	Y – intercept (V)	r^2
3	0.0247	-0.0329	0.9976
5.6*	0.0219	-0.0485	0.9968
7.3	0.0230	-0.0416	0.9964
11.1	0.0184	-0.0813	0.9937

* Not adjusting pH value

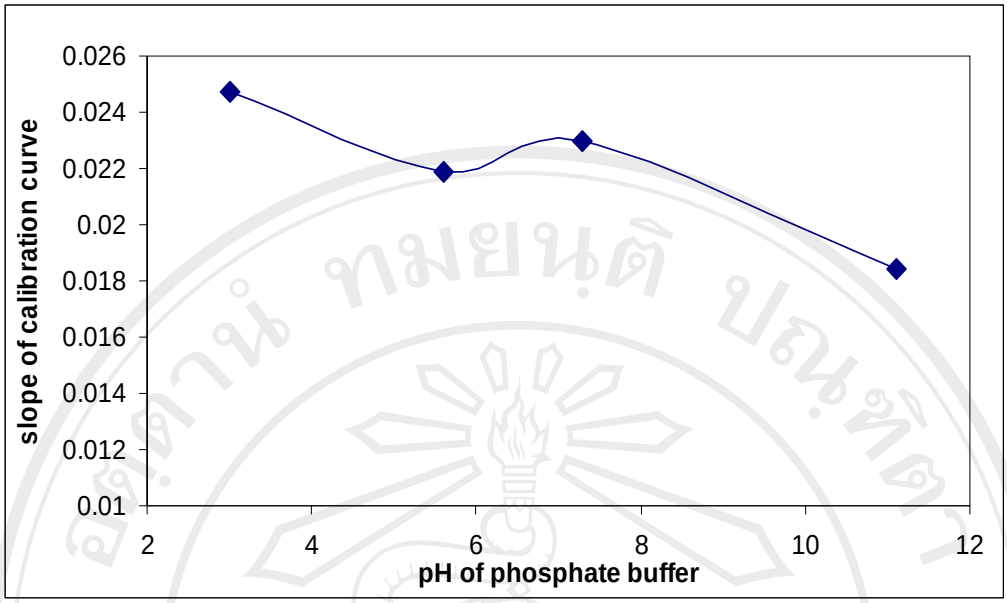


Figure 3.15 Effect of phosphate buffer pH.

With increasing of the pH, slope of the calibration graphs slightly decreased. Therefore, buffer of pH 5.6 was chosen because this solution gave high sensitivity and was easy to prepare.

3.2.1.4 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. Calibration graph data for each phosphate buffer flow rate is are summarized in Table 3.19. Slope of the calibration graphs are plotted versus flow rate of the phosphate buffer, as illustrated in Figure 3.16. It was found that the higher flow rate of the carrier stream, the higher slope of the calibration graph was obtained. The flow rate of 1.5 mL/min was chosen because it provided high sensitivity and sample throughput (20 injection per hour) with low reagent consumption.

Table 3.19 Calibration graph data for each phosphate buffer flow rate.

Phosphate buffer flow rate (ml/min)	Calibration graph data		
	Slope (V/mgL^{-1})	Y – intercept (V)	r^2
0.5	0.0132	-0.0515	0.9995
1.5	0.0209	0.0407	0.9946
2.6	0.0227	-0.0184	0.9980
4.8	0.0250	-0.0116	0.9984

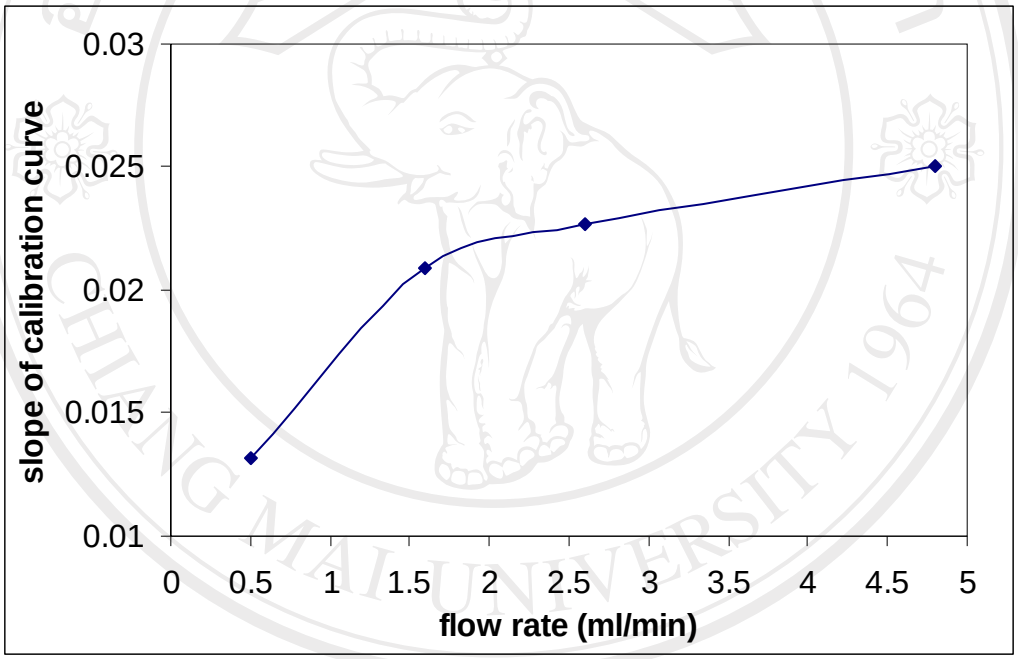


Figure 3.16 Effect of phosphate buffer flow rate.

3.2.2 FI Amperometric System with Dialysis Unit

The dialysis unit was used to separate ascorbic acid from other matrices in sample[68] when it is passing from the donor stream through a dialysis membrane into the acceptor stream. A calibration graph in concentration range of 50 – 500 mg/L was constructed by plotting peak current vs. ascorbic acid concentration. Slope, intercept and r^2 of the calibration graphs was obtained as shown in Figure 3.17

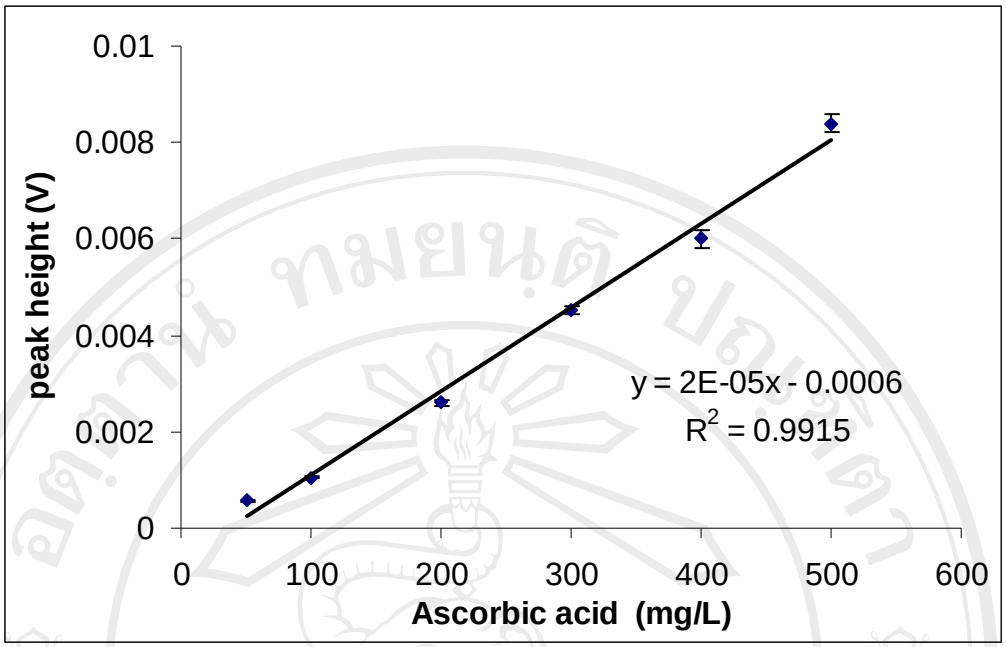


Figure 3.17 The calibration graph of ascorbic acid using dialysis FI – amperometric method.

It was found that dialysis unit gave a good calibration graph. Due to low dialysis efficiency (about 0.1%), decrease in sensitivity was observed. However, it is still useful for determination of ascorbic acid in pharmaceutical and fruit juice samples, where high concentration of ascorbic acid is concerned. By on-line dialysis, dilution factor of about 1000 could be reproducibly achieved.

3.2.2.1 Analytical characteristics of the developed method

Using the recommended conditions as summarized in Table 3.20, a linear calibration graph in the range 50 – 800 mg/L ascorbic acid was obtained as shown in Figure 3.18. Detection limit calculated from the calibration data 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 (as in Table 3.21), indicated a good precision of the method. Each injection consumed about 5 ml of reagent and 150 µl of sample.

Table 3.20 Condition for determination of ascorbic acid by dialysis flow injection amperometry.

Parameters	**Condition**
Electrodes	Working electrode : Glassy carbon electrode Auxiliary electrode : stainless steel Reference electrode : Ag/AgCl
Flow rate Donor stream Acceptor stream	 Water 1.5 mL/min Phosphate buffer 1.5 mL/min
Apply potential	800 mV
Phosphate buffer concentration	0.1 M
pH Phosphate buffer	5.6
Sample volume	50 µL

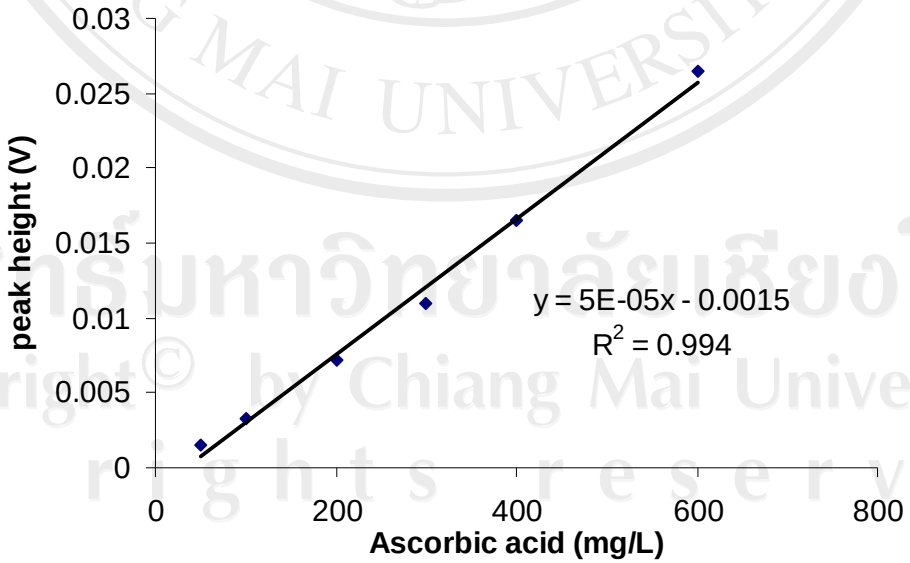


Figure 3.18 Calibration graph of ascorbic acid standard by dialysis flow injection amperomtric system.

The precision of the method was evaluated by injecting 11 replicates of 50 mg/L standard ascorbic acid, using dialysis FI – amperometry system and condition in Table 3.20. The results are shown in Table 3.21.

Table 3.21 Precision of ascorbic acid determination by dialysis FI – amperometry system

Number of injection	**Peak height (V)**
1	0.040
2	0.040
3	0.041
4	0.039
5	0.040
6	0.039
7	0.040
8	0.040
9	0.040
10	0.040
11	0.039
Mean	**0.040**
SD	**6.03×10^{-4}**
% RSD	**1.51**

3.2.2.2 Application of the developed method to fruit juice samples

The optimized system was applied to the determination of ascorbic acid in locally available commercial fruit juice samples. The preparation of sample solution was described in section 2.2.2. Ascorbic acid contents determined by FI-amperometric method, titrimetric standard method [69, see Appendix C], and labeled values are shown in Table 3.22. FIAgrams are depicted in Figure 3.19. The results have high variation which maybe due to unstability of ascorbic acid or the present of interferences in samples.

Table 3.22 Determination of ascorbic acid in fruit juice samples.

No.	Brand	Type	Ascorbic acid found (mg/L)		
			FI – amperometric method	Titrimetric method	label
1	Malee	Sweet Orange juice	292±6.1	131	-
2	Malee	Yusu Orange juice	337±4.2	120	-
3	Malee	Navel Orange juice	280±2.2	44.4	480
4	Malee	Guava juice	244±0.6	67.6	-
5	Malee	Apple juice	337±7.3	154	-
6	Malee teen	Orange mixed Carrot juice	118±3.8	4.94	75
7	Malee teen	Blueberry mixed Apple juice	188±1.7	9.87	180
8	Malee teen	Orange mixed Banana juice	247±2.6	14.8	180
9	Tipco	Tangerine juice	587±4.2	356	330
10	Tipco	Sai Nam Phueng Orange juice	563±3.9	418	-
11	Tipco	Shogun Orange juice	488±3.0	334	420
12	Tipco	Guava juice	554±4.0	424	360
13	Tipco	Kiwi and Grape juice	515±18.0	388	480
14	Tipco	Pineapple juice	549±4.2	277	90
15	Tipco	Broccoli and Fruit juice	440±16.2	321	-

Table 3.22 Determination of ascorbic acid in fruit juice samples (continued).

No.	Brand	Type	Ascorbic acid found (mg/L)		
			FI – amperometric method	Titrimetric method	label
17	Malee teen	Orange juice with sacs	145±0.3	11.1	25
18	Malee teen	Apple juice	83.7±0.1	7.22	-
19	Malee teen	Pineapple juice	351±6.7	72.2	-
20	Malee teen	Lychee juice	96.4±0.4	2.41	-
21	UFC refresh	Orange juice	415±7.7	280	-
22	Chabaa	Orange juice with sacs	182±0.9	28.9	-
23	Tropicana twister	Orange juice	334±6.0	132	240
24	Oishi Seiki	Orange juice	658±9.9	494	1594

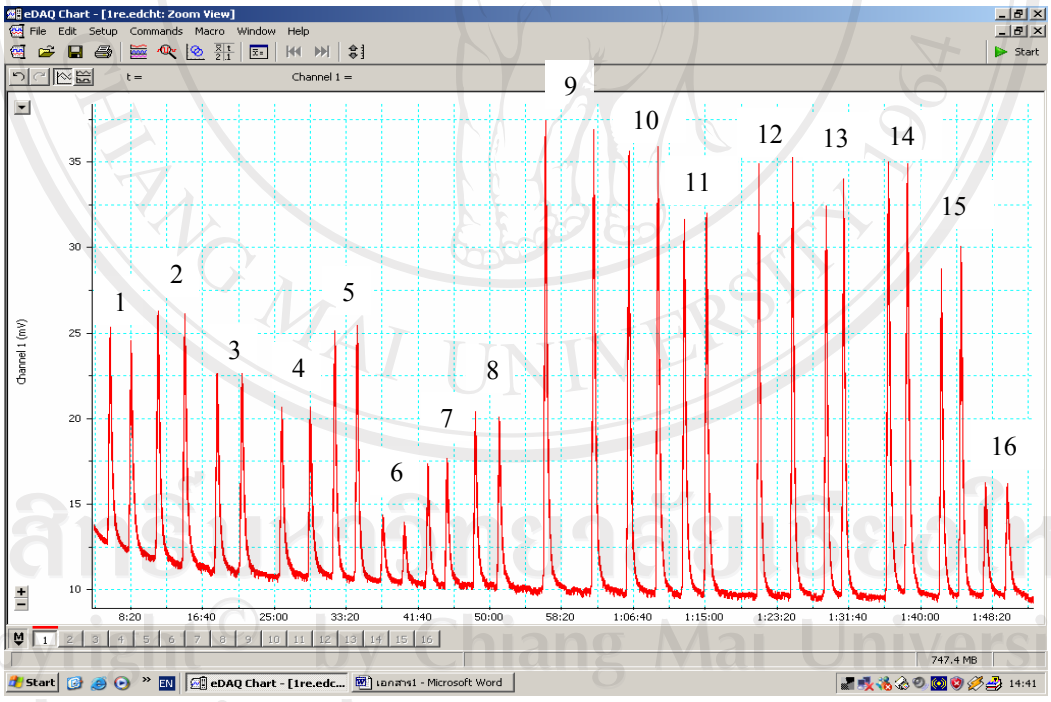


Figure 3.19 FI gram of ascorbic acid in fruit juice samples.

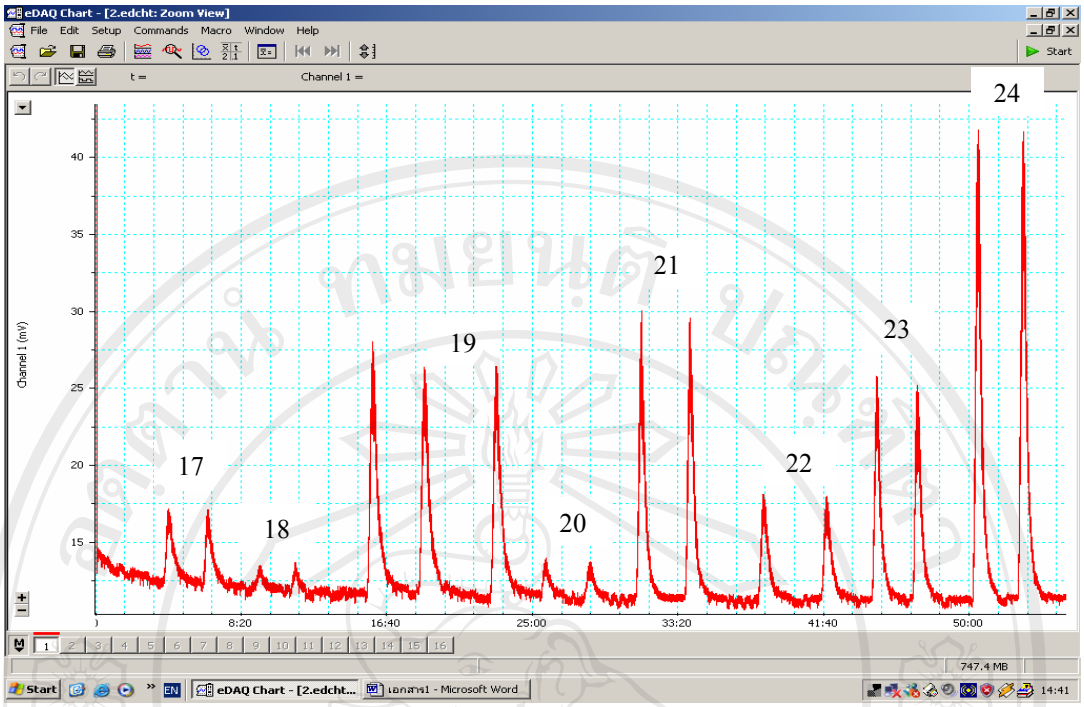


Figure 3.19 FI gram of ascorbic acid in fruit juice samples (continued).

From Table 3.23, ascorbic acid contents found in fruit juice samples by using FI-amperometric method are not correlated well with those found by the titrimetric standard method. It should be noted that the titrimetric method for fruit juice analysis is strongly interfered by colored compounds presenting in samples and may lead to a huge errors as well.

3.2.2.3 Application to vitamin C tablet samples

The vitamin C tablet samples form local drugstores were analyzed for ascorbic acid by the proposed method. All samples were prepared as described in section 2.2.2. Concentration of ascorbic acid in the sample solution was calculated from peak signal obtained using a calibration graph as shown in Figure 3.20 and Figure 3.21. Accuracy of the proposed method was determined by comparing results from FI-amperometric method with those from voltammetric method [70](see session 2.3.1.1 and Appendix D). The voltammetric method was selected for comparison

because samples contained intense colored substances that may interfere in titration. The results are shown in Table 3.23 and Figure 3.22.

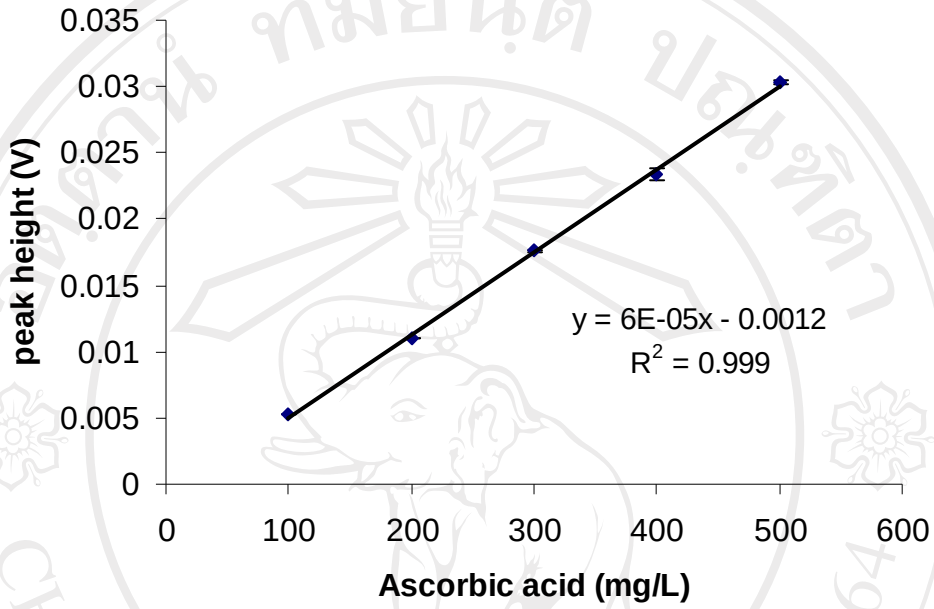


Figure 3.20 Calibration graph for ascorbic acid determination in pharmaceutical

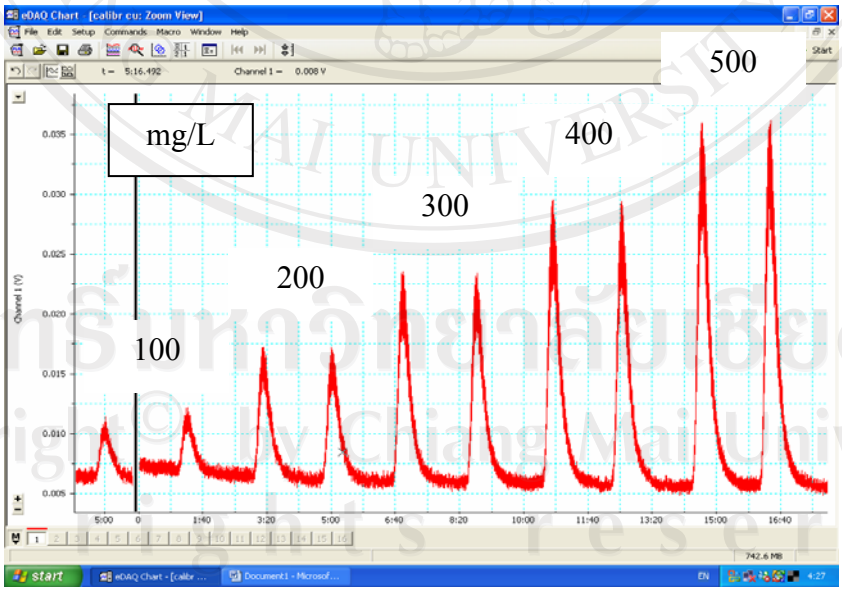


Figure 3.21 FI gram of a standard calibration curve.

Table 3.23 Determination of ascorbic acid in pharmaceutical tablet samples

No.	Sample	Label (mg/tablet)	Ascorbic acid found (mg/tablet)				% Different[c]
			FI – amperometry method [a]	% label$_1$	Voltammetric method [b]	%label$_2$	
1	Bio C 1000	1000	1021±6.0	102	1143±23	114	-11
2	Nat C	1000	998±15	100	1018±15	102	-2.0
3	Berocca C - 500	500	487±3.2	97	539±24	107	-9.6
4	nopparat	500	491±13	98	588±24	117	-16
5	Mag - C 500	500	525±7.9	105	615±15	123	-15
6	Flavettes	250	245±23	98	316±13	126	-22
7	Vitacimin	100	97±1.1	97	117±2.2	117	-17
8	Vit C 50 Frx	50	49±0.2	98	58±1.2	116	-16

[a] = duplicate results

[b] = triplicate results

[c] % different = [(FIA value – Voltammetric value)x100] / Voltammetric value

% label$_1$ = [(FIA value x 100) / label value]

% label$_2$ = [(Voltammetric value x 100) / label value]

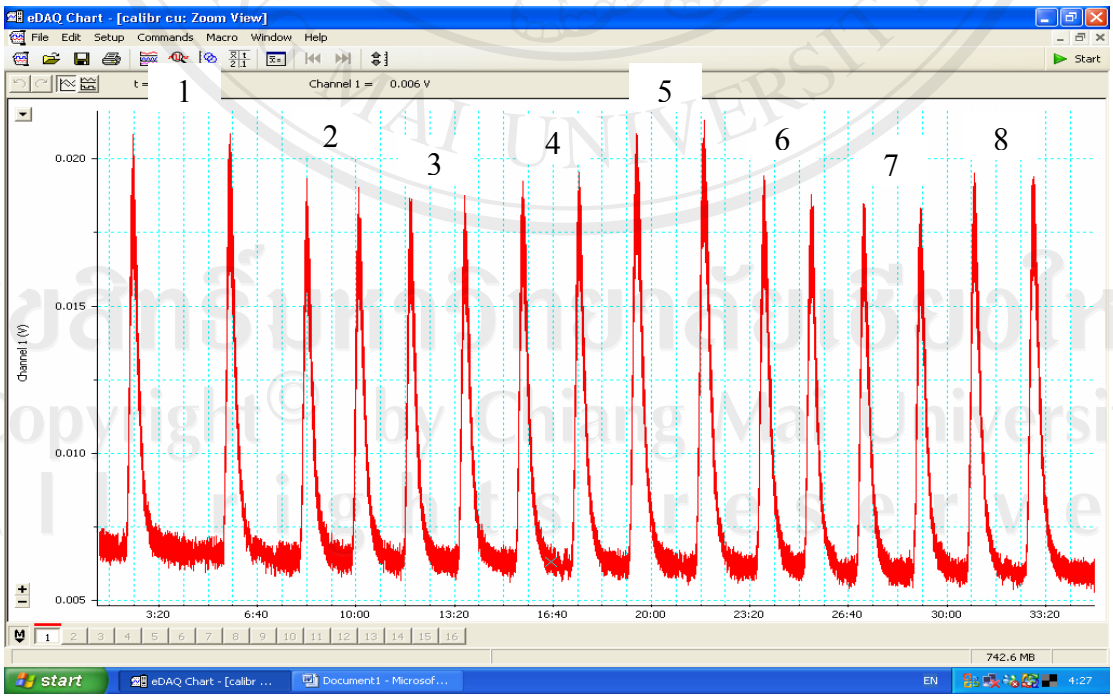


Figure 3.22 FI gram of ascorbic acid in pharmaceutical tablet samples.

From Table 3.23 it was found that the results obtained by voltammetric method had a more positive bias than those obtained by FI-amperometric method (as shown in Figure 3.23) because of the interference of other ingredients such as multivitamins and sugars. This problem is avoided by using a dialysis pretreatment FI-amperometric system.

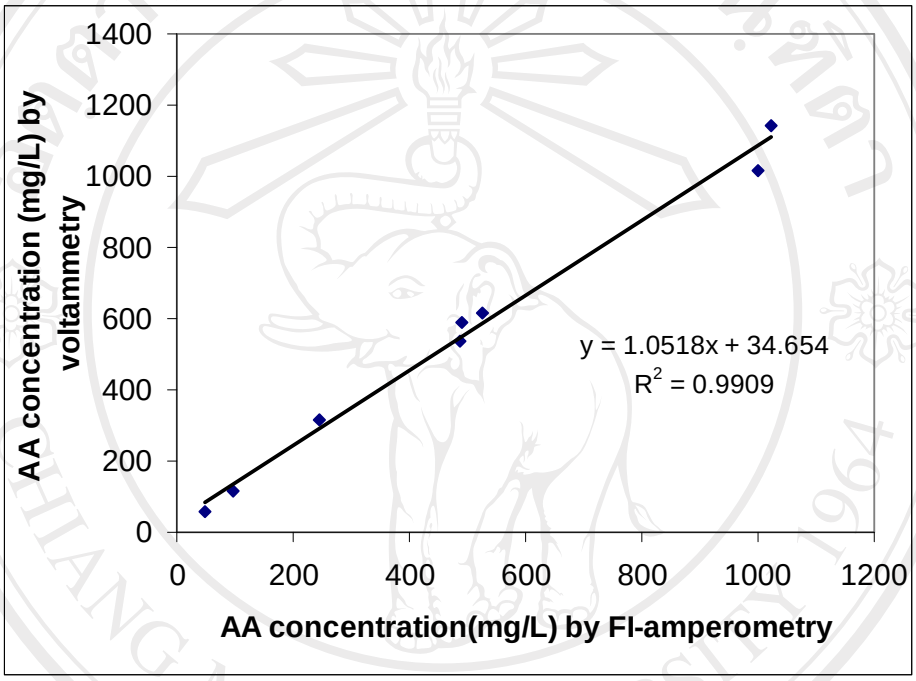


Figure 3.23 Correlation graph of ascorbic acid in pharmaceutical tablet samples by proposed method and voltammetric method.

Dialysis pretreatment flow injection amperometric method for ascorbic acid determination has more advantages than voltammetric and titrimetric methods. Chemical reagents were used in very small amounts. In addition, this technique is simple, low cost, provide good sensitivity and rapidity.