CHAPTER 3

RESULTS AND DISCUSSION

3.1 Detection reaction of the stopped-flow system for determination of vitamin C in some fruits

The stopped-flow system (Figure 2.1) was developed for increasing the sensitivity of vitamin C determination. The vitamin C in some fruits was determined based on molybdenum blue method. Vitamin C (ascorbic acid) is employed for reducing heteropoly complex obtained from the reaction of sodium molybdate and phosphate ion in acid medium, as the following reaction (1) and (2).

PO_4^{3-} + Acidic molybdate $\longrightarrow$ Heteropoly complex (yellow) --- (1)

Heteropoly complex + Ascorbic acid $\longrightarrow$ Molybdenum blue (blue) --- (2)

The preliminary parameters should be set for optimization of conditions of the system. Therefore, the merging time of standard and reagent will be studied first parameter.

Switching time of V2 and travel time of between V1 to MC2 after reagent pumping to system were studied by using dye solution and detection by visualization. The first step, the blue color solution in reagent reservoir was pumped into the system by switching V1. After 7 second, the red color solution was injected into the system by a six-port valve (V2) and inserted to the blue zone at Y-junction. Mixed color solution

was passed though the stopping coil (MC2) at 35 second. From the results, the times were setup for preliminary parameters (Table 3.1) for future study.

Table 3.1 The preliminary parameters of the stopped-flow system

Parameters	**Value**
Injection volume	100 μL
Switching time for standard/sample (V2)	7 sec.
Switching time for reagent stream (V1)	14 sec.
Travelling time of reaction zone (V1 to MC2)	35 sec.
Stopping time in stopping coil (MC2)	2 min.
Sodium molybdate	0.34×10^{-1} M
Potassium dihydrogen phosphate	0.28×10^{-1} M
Sulfuric acid (reagent and carrier)	0.46×10^{-1} M
Flow rate	2.5 mL min^{-1}
Wavelength	680 nm

3.2 Parameters study

3.2.1 Effect of travelling time on the reaction

Travelling time is the time duration that the standard and reagent combine while flowing before being stopped in the stopping coil. The travelling times (20, 22, 24, 26, 28, 30, 32, 34, 36, 38 and 40 seconds) were studies using 200 mg/L ascorbic acid and other parameters were fixed as listed in Table 3.1. The peak height and peak

area of signal were plotted versus time for traveling of the reagent zone. The results are shown in Figure 3.1-3.2 and Table 3.2, respectively.

The results show that the signal increased with increasing the travel times. The high signals were produced by the travelling time 34 to 38 seconds. It means that the reaction zone was filled the stopping coil (MC2). Travelling time at 35 second was selected for fuller studies.

Table 3.2 The effect of travelling time on peak height and peak (mean of duplicate injections)

Time (second)	**Peak height of stopped-flow gram (mV)**	**Peak area of stopped-flow gram**
20	1.19×10^2	9.09×10^4
22	2.24×10^2	1.40×10^4
24	2.33×10^2	1.39×10^4
26	2.74×10^2	1.53×10^4
28	2.62×10^2	1.39×10^4
30	2.66×10^2	1.35×10^4
32	3.08×10^2	1.39×10^4
34	3.66×10^2	2.16×10^4
36	3.48×10^2	1.87×10^4
38	3.88×10^2	1.86×10^4
40	3.56×10^2	1.52×10^4

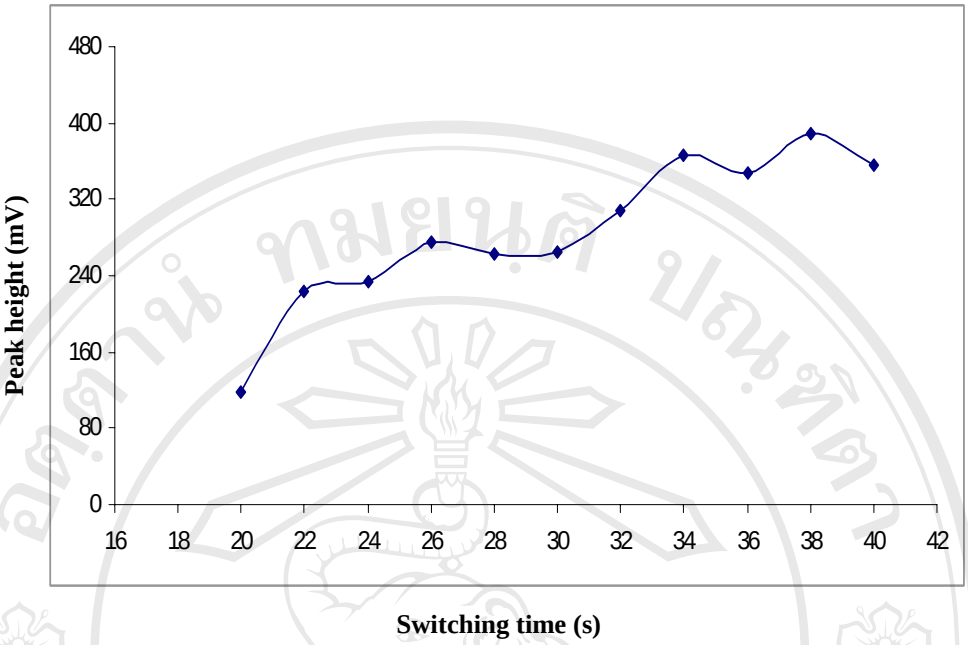

Figure 3.1 Effect of travelling time on peak height

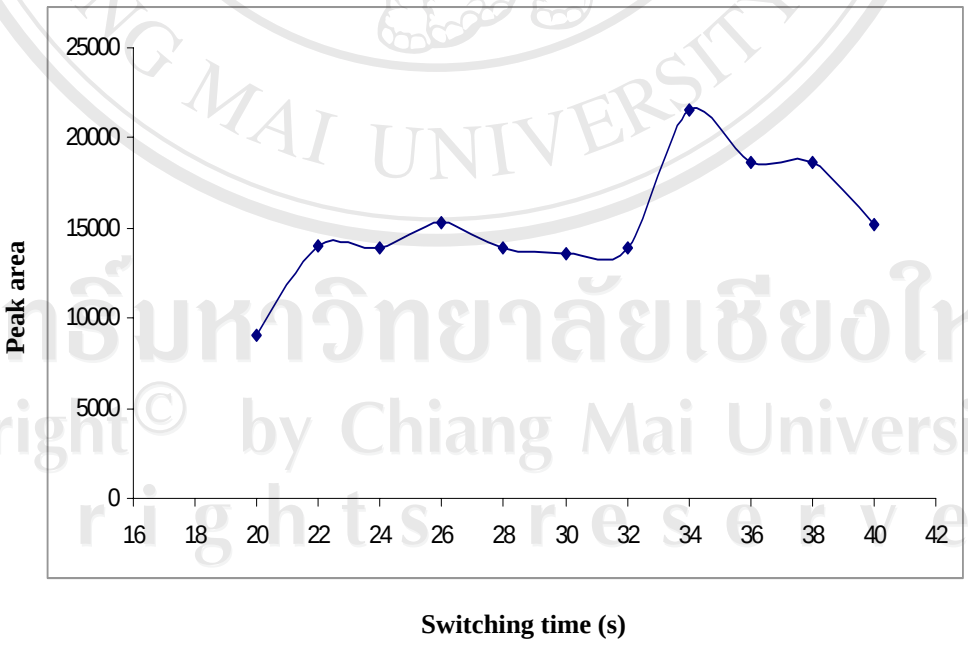

Figure 3.2 Effect of travelling time on peak area

3.2.2 Effect of reagent volume

For cost effective reason, variation of the volume of the reagent zone was investigated by varying interval of switching time of V1 to be 10, 12, 14, 16 and 20 second (133, 160, 187, 213 and 267 μL) A 100 mg/L ascorbic acid was injected into the system. The peak height and peak area of signal were plotted versus time for each plug zone of reagent. The results are shown in Figure 3.3-3.5 and Table 3.3, respectively.

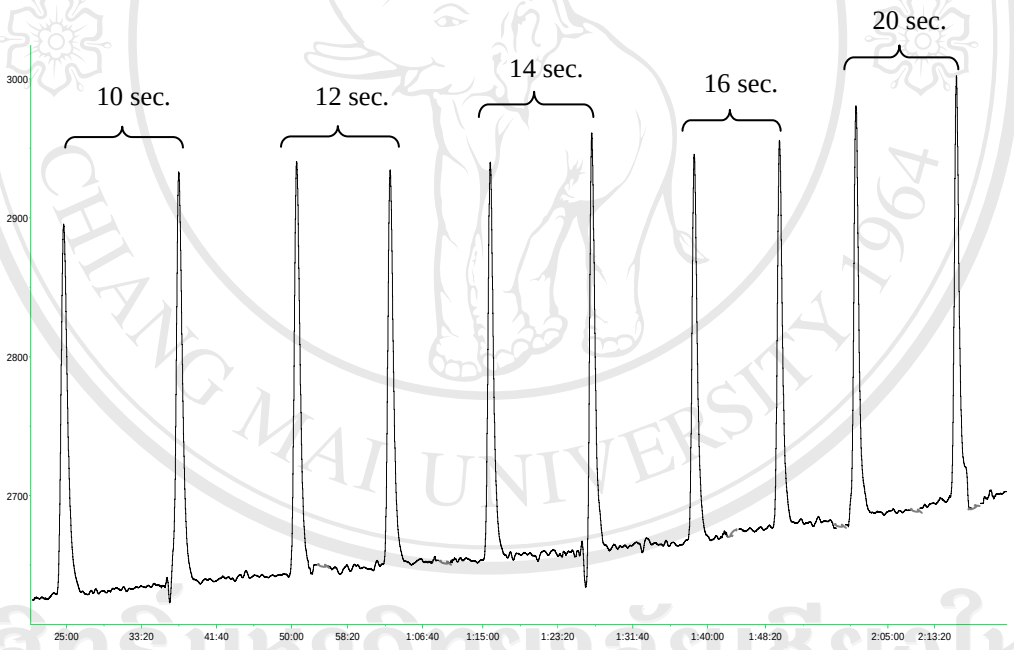

Figure 3.3 Stopped-FIA-gram of various volume of reagent stream obtained from the system

Table 3.3 Peak heights and peak area obtained from variation switching times for reagent stream (mean of duplicate injections)

Time (second)	Peak height of stopped-flow gram (mV)	Peak area of stopped-flow gram
10	2.98×10^2	1.69×10^4
12	3.06×10^2	1.57×10^4
14	3.19×10^2	1.52×10^4
16	2.99×10^2	1.41×10^4
20	3.17×10^2	1.32×10^4

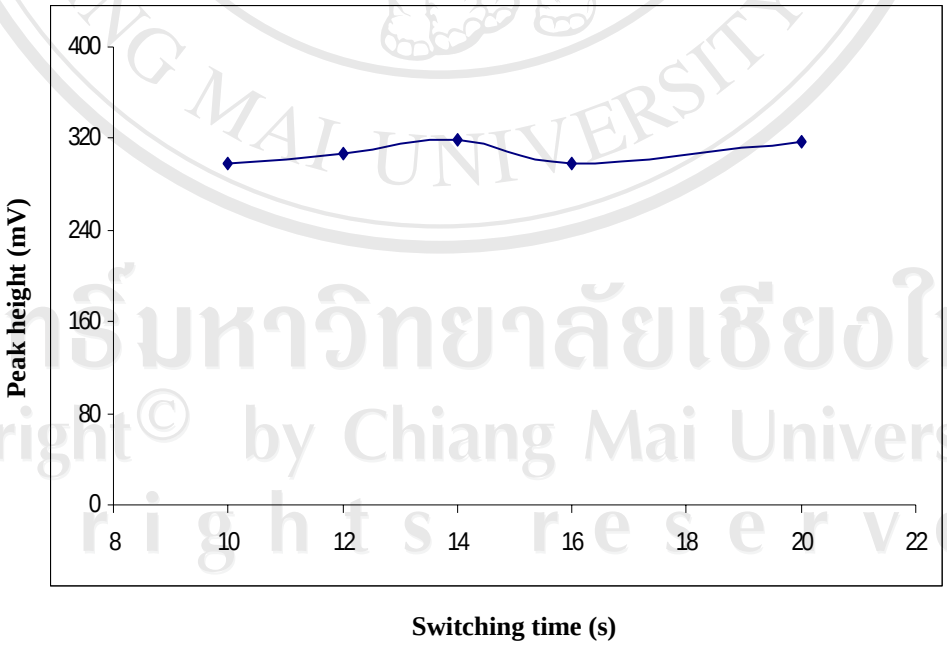

Figure 3.4 Effect of switching time of reagent stream on peak height

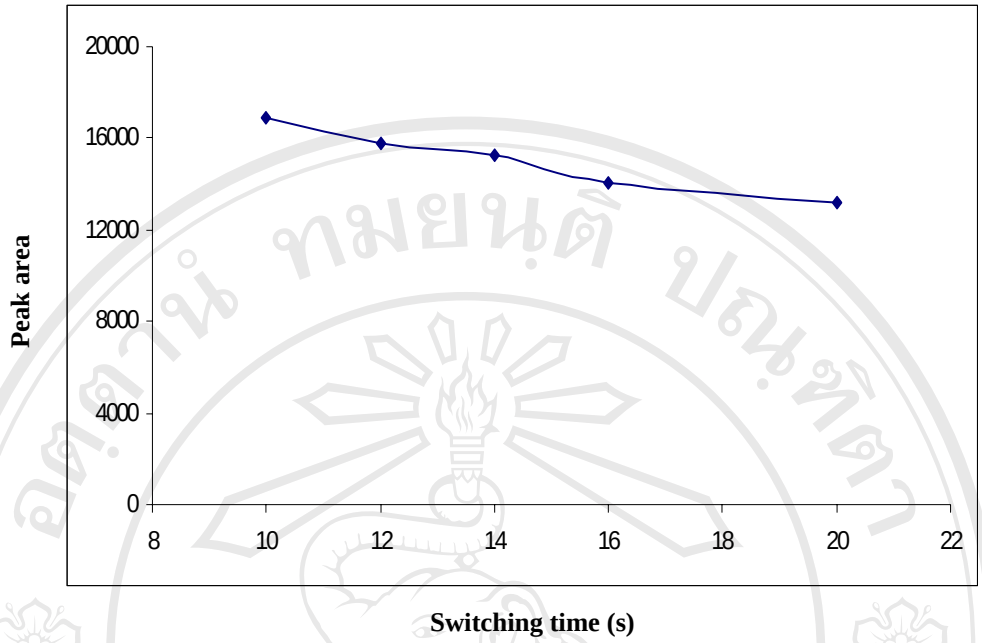

Figure 3.5 Effect of switching time of reagent stream on peak area

From the graphs of peak height and peak area, the results showed that the volumes of reagent were not significantly affected the molybdenum blue product. Thus, the switching time of reagent stream at 12 second was chosen for future studied.

3.2.3 Effect of reagent concentration

The reaction rate was depended on concentrations of ascorbic acid and reagent which consisted of sodium molybdate, potassium dihydrogen phosphate and sulfuric acid. Therefore, the effect of reagent concentration should be studied.

3.2.3.1 Effect of sodium molybdate concentration

The sodium molybdate concentration was varied from 0.34×10^{-1} to 3.40×10^{-1} M, while potassium dihydrogen phosphate and sulfuric acid concentrations

were fixed at 0.28×10^{-1} and 0.92×10^{-1} M, respectively. A series of standard ascorbic acid solutions (20-100 mg/L) was injected, with the switching time for standard/sample (V2), switching time for reagent stream (V1), travelling time of reagent zone (V1 to V3) and stopping time in stopping coil (MC2) were fixed at 7 second, 12 second, 35 second and 5 minute, respectively. Calibration graph data obtained from the study were summarized in Table 3.4 and 3.5 and slope was chosen for plotting against the concentration of ascorbic acid.

Table 3.4 The effect of sodium molybdate concentrations (mean of triplicate injections)

Concentration of sodium molybdate ($\times10^{-1}$ M)	**Peak height (mV)**				**Peak area**			
	Ascorbic acid (mg/L)				**Ascorbic acid (mg/L)**			
	20	40	60	100	20	40	60	100
0.34	11.0	32.2	77.0	146.9	641	1711	3176	5147
0.68	25.4	53.4	118.5	167.8	1063	1747	3915	6206
1.36	18.9	58.8	130.3	147.4	738	2864	5056	5184
2.04	46.2	112.0	171.2	262.8	1581	5240	6869	9407
3.40	27.6	44.4	106.3	221.0	746	1085	3490	8598

Table 3.5 Calibration graph data using varing sodium molybdate concentrations.

Concentration of sodium molybdate ($\times 10^{-1}$ M)	Calibration graph data of peak height (mV)		Calibration graph data of peak area	
	y = ax + b	r^2	y = ax + b	r^2
0.34	y = 1.75 X – 29.4	0.9905	y = 47.7 X + 5.22×10^2	0.9758
0.68	y = 1.84 X – 10.1	0.9618	y = 57.1 X + 6.61×10^2	0.9866
1.36	y = 1.64 X – 1.34	0.8606	y = 43.4 X + 1.50×10^3	0.6969
2.04	y = 2.68 X + 0.59	0.9917	y = 92.4 X + 6.87×10^2	0.9280
3.40	y = 2.52 X – 39.1	0.9711	y = 103 X – 2.20×10^3	0.9500

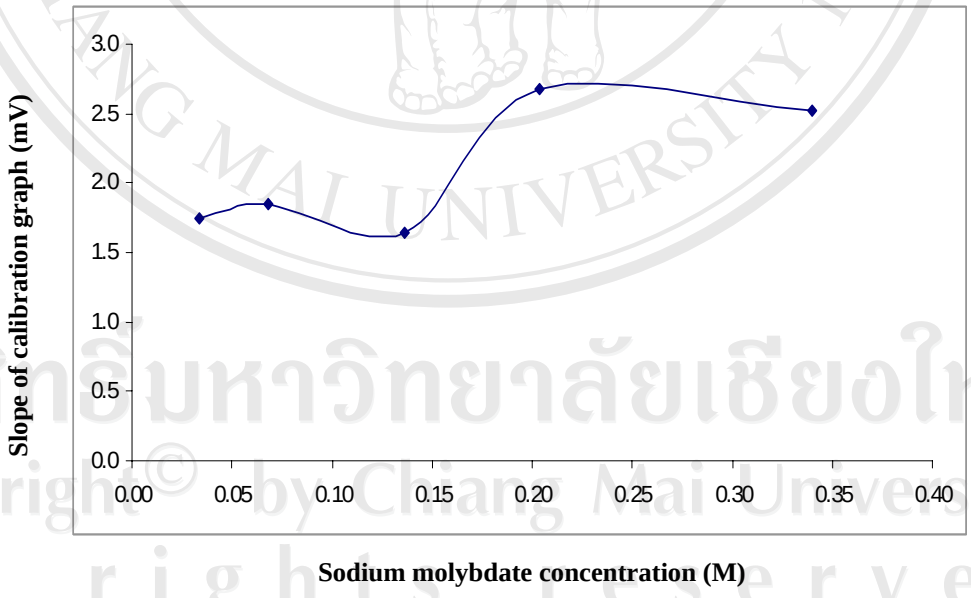

Figure 3.6 Effect of sodium molybdate concentrations on peak height

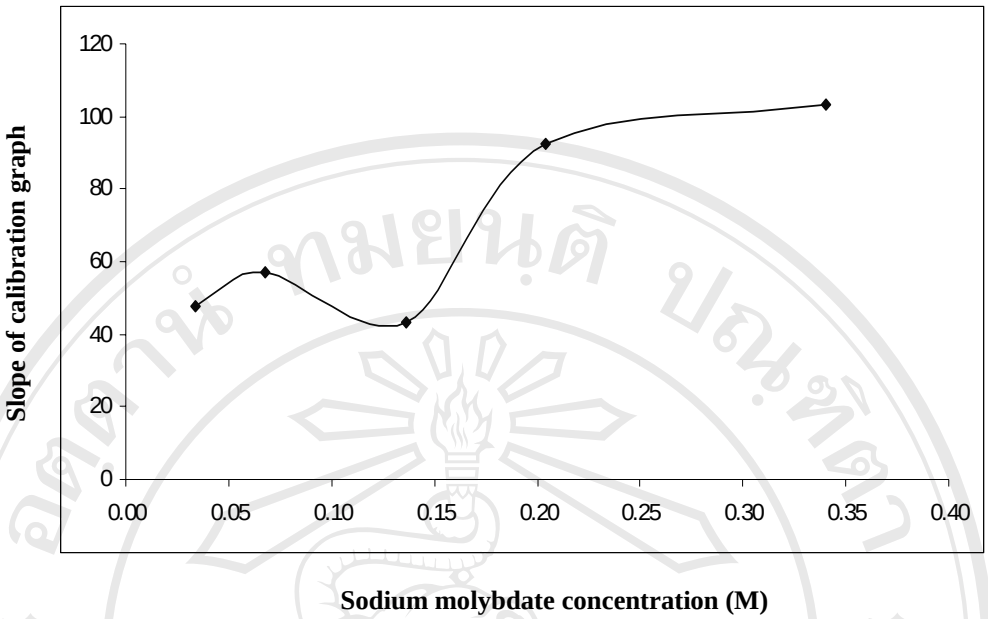

Figure 3.7 Effect of sodium molybdate concentrations on peak area

The graphs show that the slope was increased with increasing molybdate concentration. Nevertheless, the slopes of 2.04×10^{-1} M and 3.40×10^{-1} M are not significantly different. It means that concentration of sodium molybdate is excess for the reaction. Then at 2.20×10^{-1} M of sodium molybdate was chosen as suitable condition.

3.2.3.2 Effect of potassium dihydrogen phosphate concentrations

The potassium dihydrogen phosphate concentration was varied from 0.07×10^{-1} to 0.42×10^{-1} M, while sodium molybdate and sulfuric acid concentrations were fixed at 2.20×10^{-1} and 0.92×10^{-1} M respectively. A series of standard ascorbic acid solutions (20-100 mg/L) was injected for construction of calibration graphs (peak height and peak area of signals versus ascorbic acid concentrations). Slope of calibration graphs

are shown in Table 3.6-3.7 and plotted versus the concentrations of potassium dihydrogen phosphate, as illustrated in Figure 3.8 and 3.9.

Table 3.6 Data of peak using different potassium dihydrogen phosphate concentrations (mean of triplicate injections)

Concentration of potassium dihydrogen phosphate ($\times 10^{-1}$ M)	**Peak height (mV)**				**Peak area**			
	Ascorbic acid (mg/L)				**Ascorbic acid (mg/L)**			
	20	40	60	100	20	40	60	100
0.07	32.4	66.6	106.7	178.4	1587	2507	3393	6550
0.14	47.6	93.7	154.1	246.9	1954	3088	5330	9365
0.28	42.5	69.6	132.3	234.3	1637	2336	3898	9137
0.42	40.8	59.5	131.3	230.3	1666	2187	3976	9213

Table 3.7 Calibration graph data using varing potassium dihydrogen phosphate concentrations

Concentration of potassium dihydrogen phosphate ($\times 10^{-1}$ M)	**Calibration graph data of peak height (mV)**		**Calibration graph data of peak area**	
	y = ax + b	**r^2**	**y = ax + b**	**r^2**
0.07	y = 1.83 X – 4.98	0.9995	y = 62.4 X + 7.53×10	0.9777
0.14	y = 2.51 X – 2.90	0.9975	y = 95.2 X – 3.04×10^2	0.9898
0.28	y = 2.47 X – 16.63	0.9877	y = 96.6 X – 1.06×10^3	0.9480
0.42	y = 2.48 X – 21.22	0.9763	y = 98.1 X – 1.13×10^3	0.9451

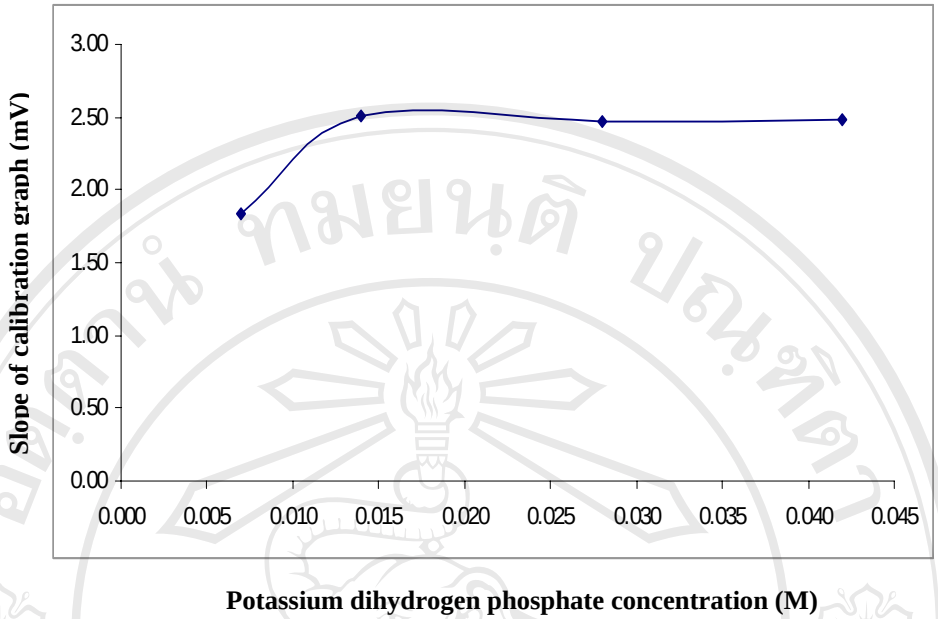

Figure 3.8 Effect of potassium dihydrogen phosphate concentrations on peak height

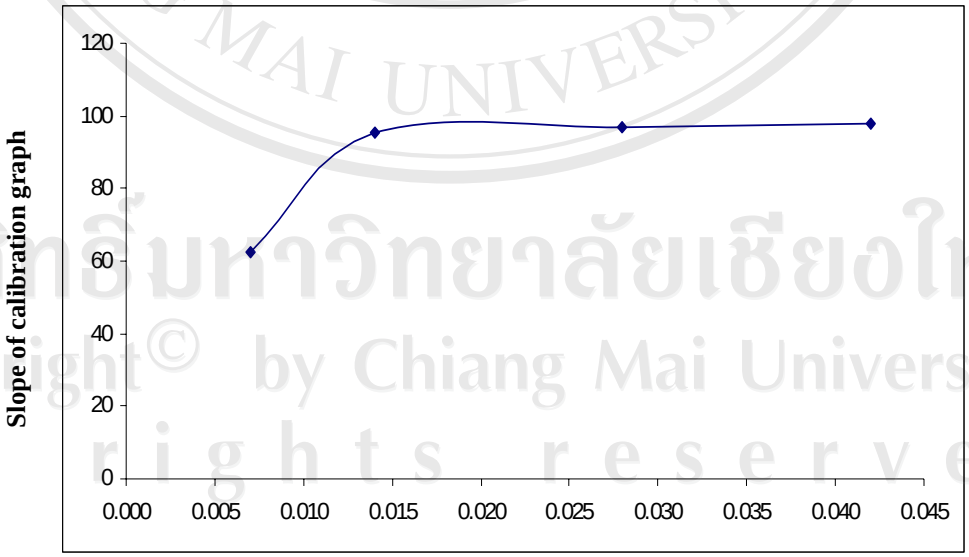

Figure 3.9 Effect of potassium dihydrogen phosphate concentrations on peak area

The slopes of the calibration graphs, obtained from peak height and peak area, indicate that although the concentration of potassium dihydrogen phosphate at 0.14×10^{-1} M and higher concentration provided much higher sensitivity than 0.07×10^{-1} M that can cause the higher concentrations of potassium dihydrogen phosphate, the reactions may occur completely. Thus, the concentration of potassium dihydrogen phosphate at 0.15×10^{-1} M was chosen for next parameter study.

3.2.3.3 Effect of sulfuric acid concentration

The sulfuric acid concentration was varied from 0.37×10^{-1} to 1.10×10^{-1} M, while sodium molybdate and potassium dihydrogen phosphate concentrations were fixed at 2.20×10^{-1} and 0.15×10^{-1} M, respectively. A series of standard ascorbic acid solutions (20-100 mg/L) was injected and calibration graph data obtained from the study were summarized in Table 3.8. A plot of the slopes versus the concentration of sulfuric acid was made as shown in Figure 3.10 and 3.11.

Table 3.8 The effect of sulfuric acid concentrations (mean of triplicate injections)

Concentration of sulfuric acid ($\times 10^{-1}$ M)	Peak height (mV)				Peak area			
	Ascorbic acid (mg/L)				Ascorbic acid (mg/L)			
	20	40	60	100	20	40	60	100
0.37	27.2	51.2	107.6	196.3	743	1205	3318	6788
0.55	42.0	95.9	157.8	265.6	1813	3311	5915	11322
0.74	39.7	86.8	136.9	239.4	1707	2929	4256	9519
0.92	38.7	67.8	124.3	224.8	1692	2387	3851	9057
1.10	33.4	59.5	118.1	216.7	1473	2132	3629	8481

Table 3.9 Calibration graph data using varing sulfuric acid concentrations

Concentration of sulfuric acid ($\times 10^{-1}$ M)	Calibration graph data of peak height (mV)		Calibration graph data of peak area	
	y = ax + b	r^2	y = ax + b	r^2
0.37	y = 2.18 X– 24.6	0.9878	y = 77.9 X – 12.7×10^2	0.9634
0.55	y = 2.81 X – 14.1	0.9993	y = 118 X – 58.8×10^2	0.9500
0.74	y = 2.50 X– 12.1	0.9997	y = 92.9 X – 6.96×10^2	0.9710
0.92	y = 2.39 X – 17.6	0.9906	y = 88.5 X – 8.25×10^2	0.9516
1.10	y = 2.36 X – 23.2	0.9878	y = 84.6 X – 8.70×10^2	0.9578

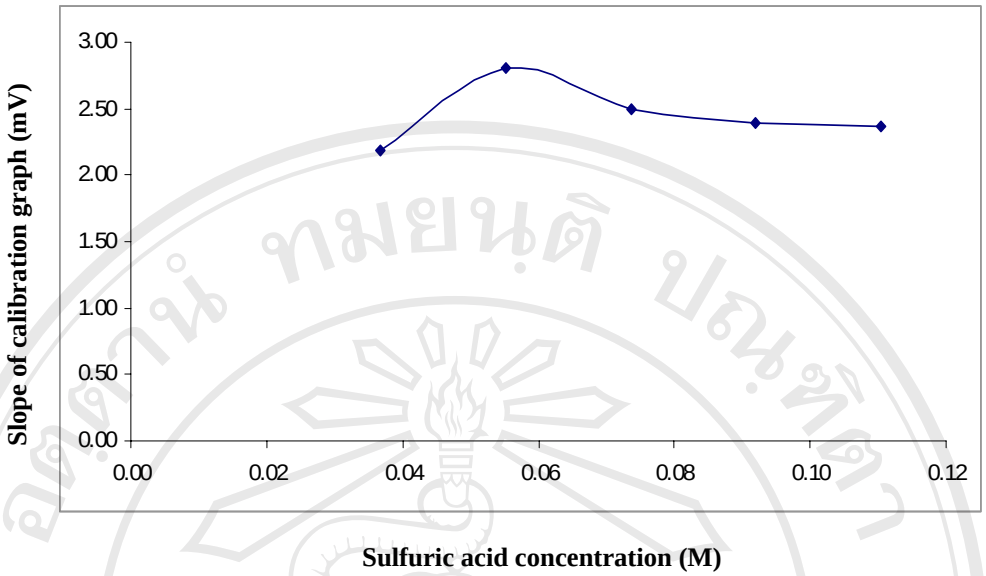

Figure 3.10 Effect of sulfuric acid concentration on peak height

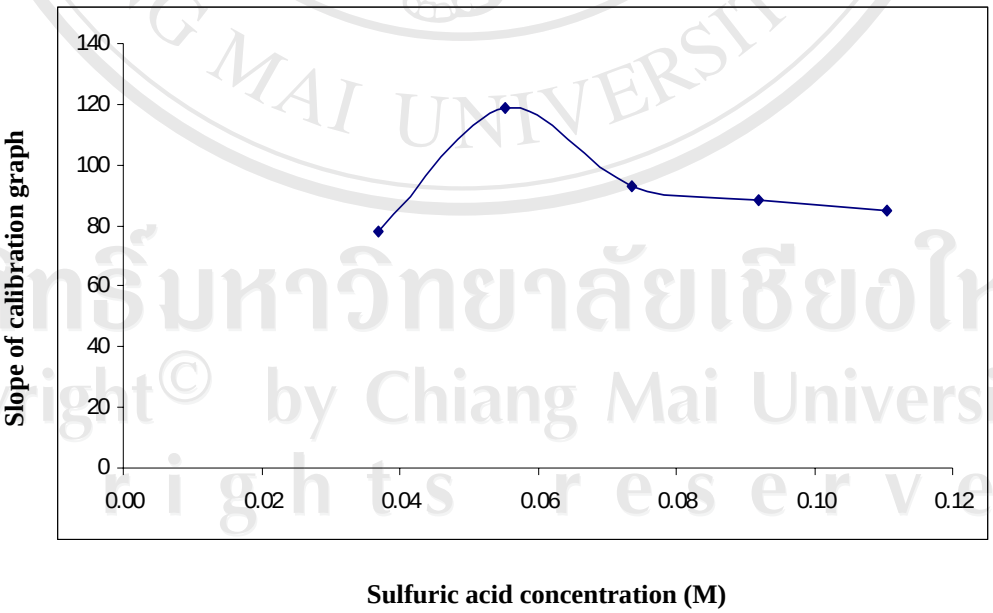

Figure 3.11 Effect of sulfuric acid concentration on peak area

The slopes of peak height and peak area at 0.74×10^{-1} to 1.10×10^{-1} M showed similar trend. But at the sulfuric acid concentrations level lower than 0.55×10^{-1} M, sodium molybdate may not be completely converted to molybdic acid. Nevertheless, the rate of reaction is slower at high concentration of sulfuric acid than at lower acid concentration. Therefore, 0.74×10^{-1} M of sulfuric acid was selected as the suitable concentration.

From the FIA gram (Figure 3.12), the small peaks appear in the front of the analytical peak when the long travelling times were used (34-35 seconds). The analytical of these peaks depend on the volume of the injected reaction zone that passed into MC2. It indicates that if travelling times was too short (31 second) the reaction zone did not fill the MC2. However, if the travelling time was too long, the portion of the reaction zone would enter the detector before the stopping time was ended. Therefore, the travelling time was re-studied to obtain the suitable time.

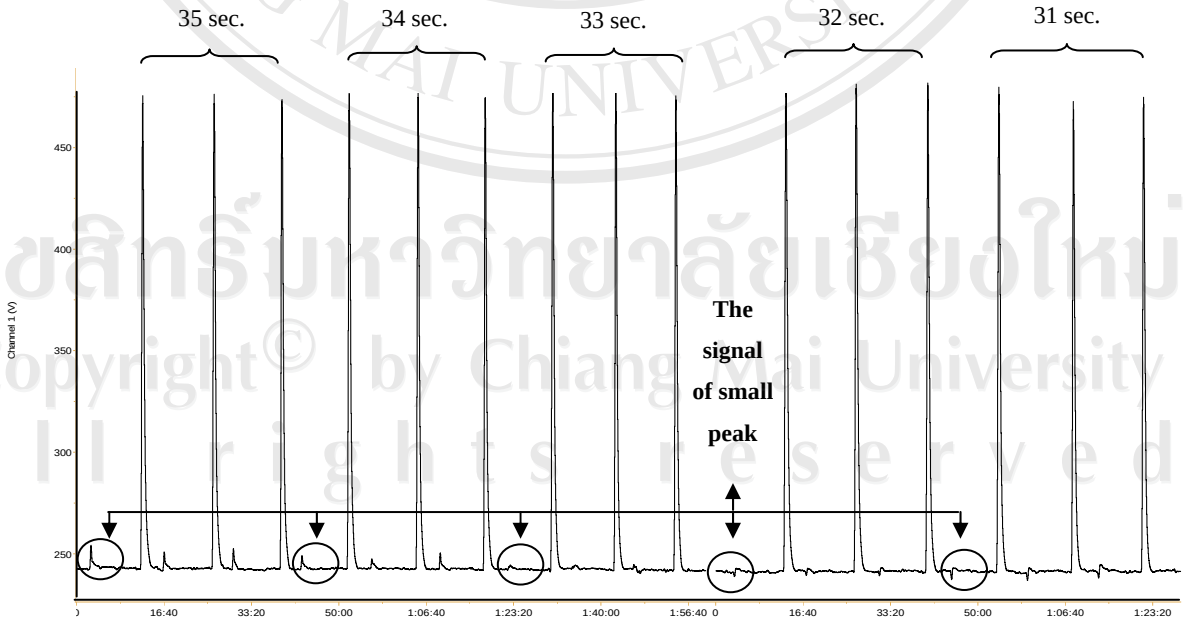

Figure 3.12 FIA gram of various travelling time

3.2.4 Re-study of travelling time

The travelling time for the reactance zone was studies and varied from 31 to 35 seconds. Concentration of sodium molybdate, potassium dihydrogen phosphate and sulfuric acid were 2.20×10^{-1} M, 0.15×10^{-1} M and 0.74×10^{-1} M, respectively. A standard ascorbic acid solution of 100 mg/L was injected and the peak height / peak area of the signals were plotted against traveling time. The results are shown in Figure 3.12-3.14 and Table 3.10, respectively.

Table 3.10 The effect of travelling time on peak height and peak (mean of triplicate injections)

Time (second)	**Peak height of stopped-flow gram (mV)**	**Peak area of stopped-flow gram**
31	$2.34\times10^{2} \pm 3.77$	$7.71\times10^{3} \pm 382.68$
32	$2.40\times10^{2} \pm 0.86$	$8.41\times10^{3} \pm 191.30$
33	$2.35\times10^{2} \pm 1.93$	$7.31\times10^{3} \pm 132.17$
34	$2.34\times10^{2} \pm 1.78$	$7.50\times10^{3} \pm 124.96$
35	$2.32\times10^{2} \pm 1.01$	$7.79\times10^{3} \pm 320.34$

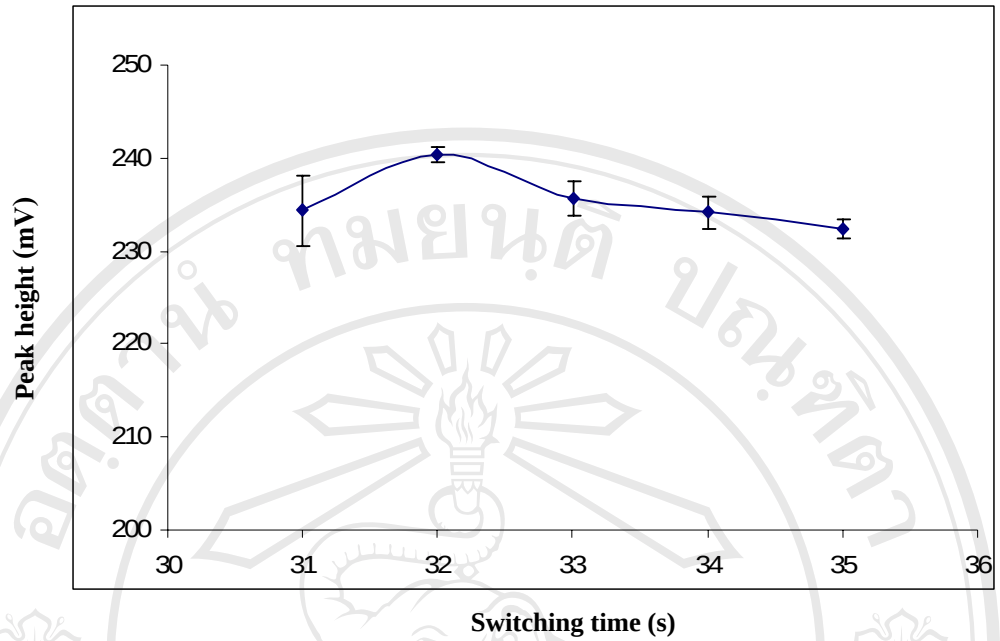

Figure 3.13 Effect of traveling time on peak height

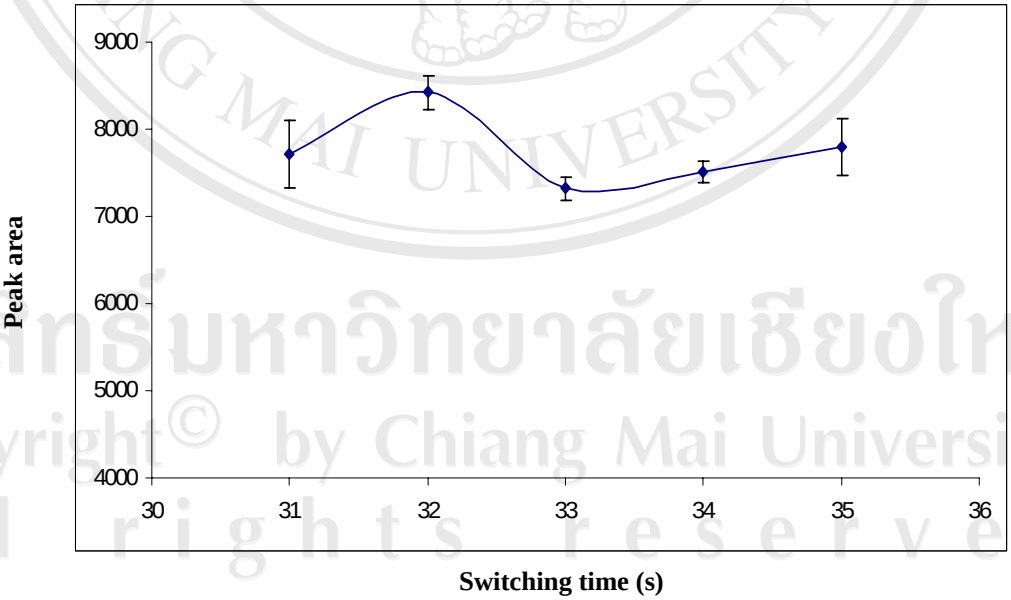

Figure 3.14 Effect of travelling time on peak area

The results shown that, the traveling time at 32 second showed no unwanted peak in front of the analytical peak. Therefore, the travelling time of 32 second was chosen for further experiments.

3.2.5 Effect of the stopped time in the stopping coil (MC2)

Due to the theory of the stopped-flow technique, the sensitivity of measurement can be increased by increasing the stopped time of the sample and reagent in the stopping coil. The stopped time at 0, 4, 5 and 6 minutes under the same effect of traveling time of reagent zone were studied. The traveling time for the reagent zone used at 32 second and a series of standard ascorbic acid solutions (20, 40, 60, 80 and 100 mg/L) were injected. The calibration graphs of standard ascorbic acid were plotted between the concentrations of ascorbic acid and either of the peak height or peak area of the FIA grams. The analytical characteristics are listed in Table 3.11 – 3.12 and Figure 3.15 - 3.16.

Table 3.11 Data of peak using various stopped times (mean of triplicate injections)

Stopping time (min.)	**Peak height (mV)**					**Peak area**				
	Ascorbic acid (mg/L)					**Ascorbic acid (mg/L)**				
	20	40	60	80	100	20	40	60	80	100
Non-stop time	0.01	0.05	0.08	0.11	0.15	0.29	4.24	7.23	10.24	13.49
4	0.05	0.10	0.15	0.21	0.25	3.28	7.56	10.94	14.87	18.12
5	0.06	0.12	0.18	0.23	0.28	4.39	9.19	13.74	18.21	21.92
6	0.07	0.12	0.17	0.22	0.27	4.69	9.31	13.00	17.03	20.49

Table 3.12 Slope of calibration graph using varing variation stopped time

Stopping time (minutes)	**Calibration graph data of peak height (mV)**		**Calibration graph data of peak area**	
	y = ax + b	**r^2**	**y = ax + b**	**r^2**
non-stop time	$y = 1.7\times10^{-3} X - 1.91\times10^{-2}$	0.9994	$y = 1.62\times10^{-1} X - 2.62$	0.9972
4	$y = 2.5\times10^{-3} X + 0.06\times10^{-3}$	0.9981	$y = 1.85\times10^{-1} X - 0.14$	0.9980
5	$y = 2.7\times10^{-3} X + 1.36\times10^{-2}$	0.9964	$y = 2.20\times10^{-1} X + 0.26$	0.9979
6	$y = 2.5\times10^{-3} X + 2.11\times10^{-2}$	0.9989	$y = 1.96\times10^{-1} X + 1.10$	0.9977

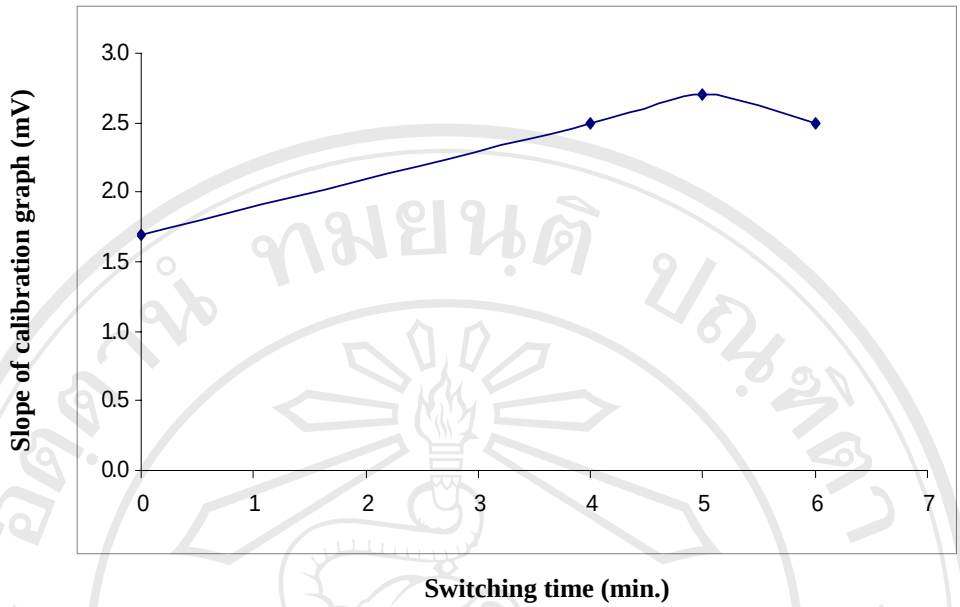

Figure 3.15 Effect of stopping time on peak height

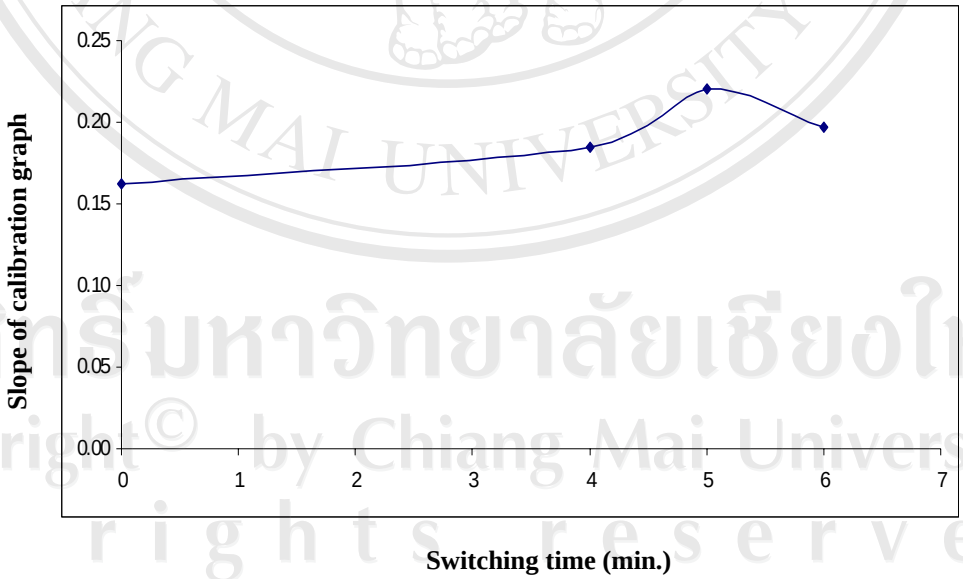

Figure 3.16 Effect of stopping time for peak area

The results indicated that the longer the stopped time (at 4 and 5 minute) tended give the high sensitivity because the intensity of the blue color product was increased when increasing reaction time. However, the stopped time at 6 minute provided the sensitivity lower than at 5 minute for the proposed system. This may be due to the diffusion effect of the stopped solution during the long period of time that can cause the lower absorbance of the same solution. Therefore, the stopped time at 5 minute was chosen for further studies.

3.2.6 Summary of the operation time in each step

Table 3.13 Operation time of the stopped flow system for vitamin C determination

Step	Time	Sequence				Description
		V1 (3-way valve)	**V2** (6-port valve)	**V3** (6-port valve)	**V4** (3-way valve)	
1	Start	Turn on to reagent	Load position	Inject position	Turn on to waste	- Flow reagent to system - Load standard / sample
2	7 sec.	Turn on to reagent	Inject position	Inject position	Turn on to waste	- Inject standard / sample to system
3	12 sec.	Turn on to H_2SO_4	Inject position	Inject position	Turn on to waste	- Stop the reagent - Flow H_2SO_4 to system
4	32 sec.	Turn on to H_2SO_4	Inject position	Load position	Turn on to waste	- Stop mixing zone (reagent + standard / sample) in mixing coil
5	1.30 min.	Turn on to H_2SO_4	Inject position	Load position	Turn on to H_2SO_4 reservoir	- Return H_2SO_4 as carrier to reservoir for reused
6	5.00 min.	Turn on to H_2SO_4	Inject position	Load position	Turn on to waste	- Turn carrier to waste before inject color product to detector
7	5.32 min.	Turn on to H_2SO_4	Inject position	Inject position	Turn on to waste	- Inject the color product to detector

3.2.7 Summary of the selected conditions

The optimum conditions of the stopped-flow system for vitamin C determination in some fruits can be summarized in the Table 3.14

Table 3.14 Conditions used for the determination of vitamin C in some fruits

Parameters	**Value**
Injection volume	100 μL
Switching time for standard/sample (V2)	7 sec.
Switching time for reagent stream (V1)	12 sec.
Travelling time of reactant zone (V1 to V3)	32 sec.
Stopping time in stopping coil (MC2)	5 min.
Sodium molybdate	2.20×10^{-1} M
Potassium dihydrogen phosphate	0.15×10^{-1} M
Sulfuric acid (reagent and carrier)	0.74×10^{-1} M and and 0.55×10^{-1} M
Flow rate	2.5 mL min^{-1}
Wavelength	680 nm

3.3 Calibration graph

A calibration data and FIA grams obtained in the range of 10-100 mg/L ascorbic acid are shown in Table 3.15 and Figure 3.17, respectively. The calibration curves obtained from either peak height or peak area (Figure 3.18-3.19) show the same trends, increasing of concentration improve the peak height or peak area. Also, the peak shape of the FIA gram as shown in Figure 3.17 is symmetrical. Therefore, the peak height was selected as the detection parameter for determination of vitamin C in fresh fruit samples. Detection limit [50] calculated from the calibration graph data was found to be 4.45 mg/L ascorbic acid.

Table 3.15 Calibration graph data of 10-100 mg/L ascorbic acid (mean of triplicate injections)

Concentration of ascorbic acid (mg/L)	**Peak height of the stopped-flow gram (mV)**	**Peak area of the stopped-flow gram (mV)**
10	22.0	1101
20	48.9	1790
40	100.1	3687
60	157.4	5177
80	199.9	6180
100	232.0	7062

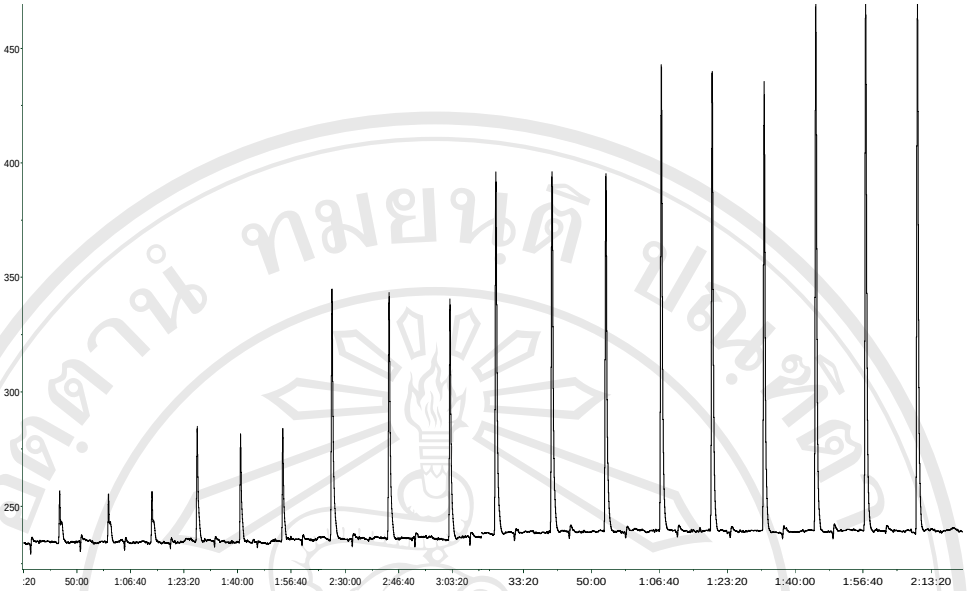

Figure 3.17 FIA-gram of various ascorbic acid standard concentrations obtained from the stopped-flow system

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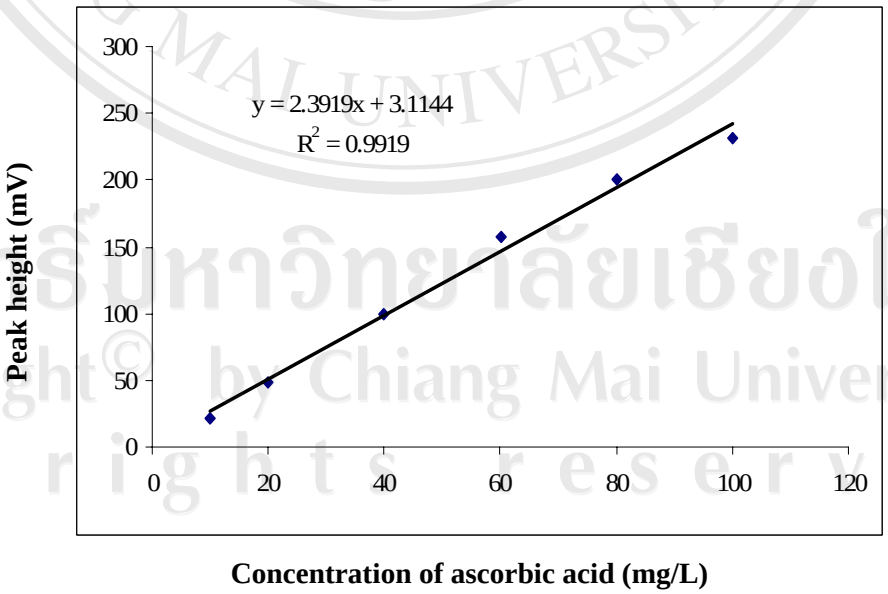

Figure 3.18 A Calibration graph of peak height for vitamin C determination obtained from the stopped-flow method

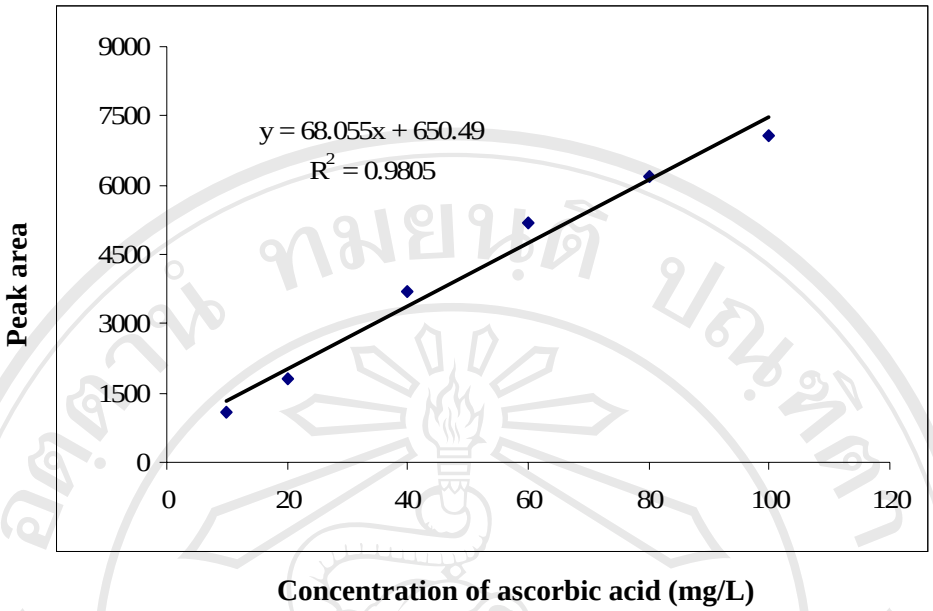

Figure 3.19 A Calibration graph of peak area for vitamin C determination obtained from stopped-flow method.

3.4 Precision

A solution containing 40 mg/L of the standard ascorbic acid was used to study precision of the proposed method. The analyses of 14 replicates were done under the selected condition. The results are shown in Table 3.16.

Table 3.16 Precision of ascorbic acid determination by stopped-flow method

Number of injection	Peak height of stopped-flow gram (mV)	Concentrations of ascorbic acid (mg/L)
1	0.1115	44.47
2	0.1065	42.55
3	0.0982	39.33
4	0.0988	39.57
5	0.1057	42.23
6	0.1115	44.46
7	0.1013	40.55
8	0.1094	43.64
9	0.1085	43.30
10	0.1032	41.28
11	0.1030	41.19
12	0.1110	44.27
13	0.1053	42.08
14	0.1065	42.52
Mean	0.1057	42.24
SD	0.0044	1.70
% RSD	4.19	4.03

From the results, it was found that the relative standard deviation (RSD) obtained was 4 %.

3.5 Interference study

Tartaric acid and its salt can be found in some fruit [51-52]. It is a chelating agent that may be bound to molybdenum. Tartaric acid may interfere the determination of vitamin C samples. A series of chelate salt at 0, 2.61×10^{-4}, 1.30×10^{-3} and 2.61×10^{-3} mole (0, 60, 300 and 600 mg/L) was added in to 3.41×10^{-4} mole (60 mg/L) of ascorbic acid. These solutions were studied for interference. The results are shown in Table 3.17.

Table 3.17 Effect of tartrate determination by stopped-flow method (mean of triplicate injections)

Peak height (mV)	**Amount of tartrate (mole) added to ascorbic acid**			
	0	**2.61×10^{-4}**	**1.30×10^{-3}**	**2.61×10^{-3}**
1	163.10	146.64	125.48	134.07
2	159.37	154.01	128.26	130.90
3	158.10	159.42	126.50	122.99
4	158.08	159.90	124.15	116.25
5	156.34	157.41	123.85	113.27
Mean	159.00	155.47	125.65	123.49
2SD	5.07			

The results show that the negative error of signal was increased with increasing tartrate concentration because the molybdenum in the reagent may be bound to tartrate. Table 3.17 shows that tartrate in the ratio of 1.0 : 0.7 moles (ascorbic acid : tartrate) can interfere ascorbic acid determination.

3.6 Determination of vitamin C in some fruit samples

Ascorbic acid in fresh fruit juice samples were determined by proposed method and titrimetric method [53]. The results shown in Table 3.18

Table 3.18 The amount of vitamin C in fresh fruit juice obtained from the stopped-flow method and titrimetric method ([a] = mean of triplicate injections)

Samples number	Fruit samples	Vitamin C in sample (mg/kg)	
		Stopped-flow method [a]	Titrimetric method
1	Mango 1	87	222
2	Mango 2	56	176
3	Apple red 1	17	10
4	Apple red 2	27	7
5	Apple fuji 1	18	13
6	Apple fuji 2	16	5
7	Apple green	18	10
8	Chinese pear 1	12	6
9	Chinese pear 2	12	15
10	Rose-apple red 1	35	27
11	Rose-apple red 2	37	47
12	Rose-apple green	22	11
13	Pineapple 1	56	42
14	Pineapple 2	66	47
15	Tangerine 1	90	103
16	Tangerine 2	68	48
17	Star fruit 1	154	188
18	Star fruit 2	176	202
19	Lemon 1	213	299
20	Lemon 2	233	240
21	Strawberry 1	394	473
22	Strawberry 2	380	478
23	Jujube 1	223	221
24	Jujube 2	406	386
25	Guava 1	599	602
26	Guava 2	2,161	2,093

The results showed that the proposed method found ascorbic acid in mango at level less than titrimetric method. This may be because tartaric acid presented in fresh mango samples.

Apple and chinese pear juices can produce red-brown color because phenolic compounds in juices were oxidized by oxygen in the air. It interfered both the titrimetric method and the proposed method. The negative error was obtained from the reference method because its color affects the end point. For the stopped flow injection system, the positive error was obtained because the color can be detected at 680 nm. Nevertheless, the error can be substrate by using blank signal.

The results (no.1 – no.26) obtained by flow based system were compared to reference method. The correlation plot (Figure 3.20) indicated that the results obtained by two methods are not significantly difference.

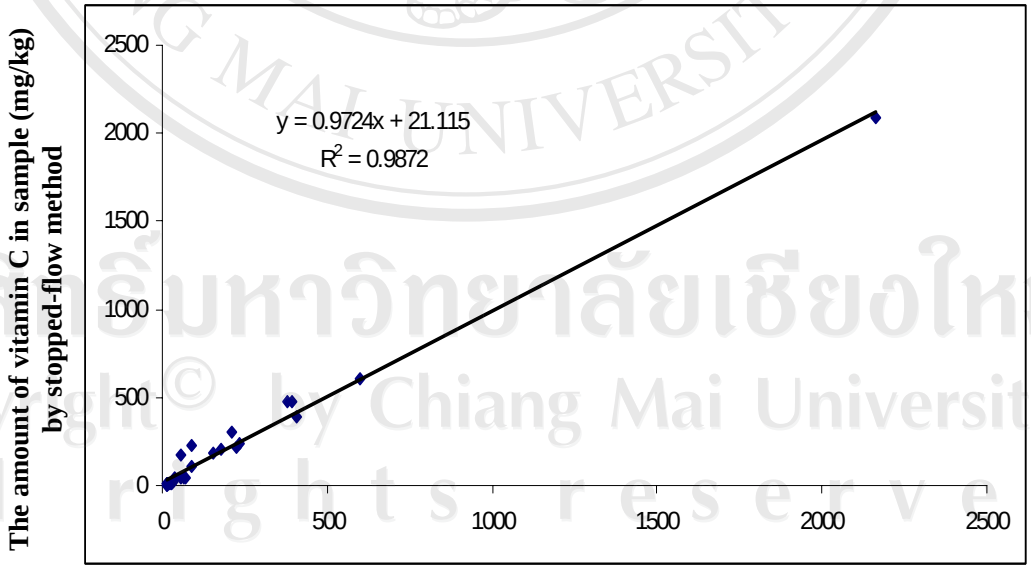

Figure 3.20 Correlation graph of the amount of vitamin C found in fruit samples by stopped-flow system and titrimetric method

However from the results, the amount of vitamin C found in some fruit samples determined by using the developed stopped-flow method agree well with those amount obtained by the titrimetric method at 95 % confident level of paired t-test. The correlation graph of amount vitamin C found in some fruit samples obtained by a stopped-flow method and a titrimetric method are shown in Figure 3.20.