CHAPTER III

RESULTS AND DISCUSSION

3.1 Preliminary conditions of SIA-LOV for determination of acidity

Sequential injection analysis-lab-on-valve (SIA-LOV) for determination of acidity involves acid-base titration. However, principle of the procedure is different from flow-injection titration as it is not based on dispersion of the titration zone. In SIA-LOV acid-base titration system, first, an acid concentration range was fixed. From the acidity found in fruit juice samples by standard titration method, which were 0.2-1.1%(w/v) express as citric acid, the citric acid concentration range of 0.2-1.2% (w/v) was selected. After that, a fixed amount of NaOH is used as a titrant. A sample, titrant and indicator were aspirated into the system. After the titrant and a suitable volume of acidic sample (in a monosegment as shown in Figure 2.2) were well mixed, the remained amount of NaOH defined pH of the final solution which was at the beginning of titration curve as shown in Figure 3.1. For different concentrations of acid samples, the different amounts of NaOH were left leading to various pH of the final solutions which can be followed by using a suitable acid-base indicator. In this work, indigo carmine was selected because its color intensity is linearly proportional to NaOH concentration in a pH range of 11.4-13.0 (Figure 3.1 and 3.2a). It is yellow in basic solution and blue in acidic solution. The blue acidic form was selected to be monitored because it was less interfered by colored substances in the fruit juice sample.

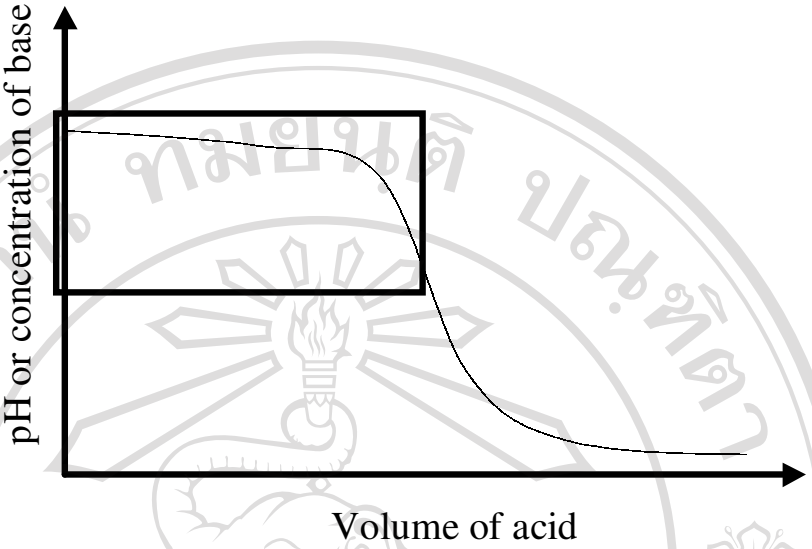
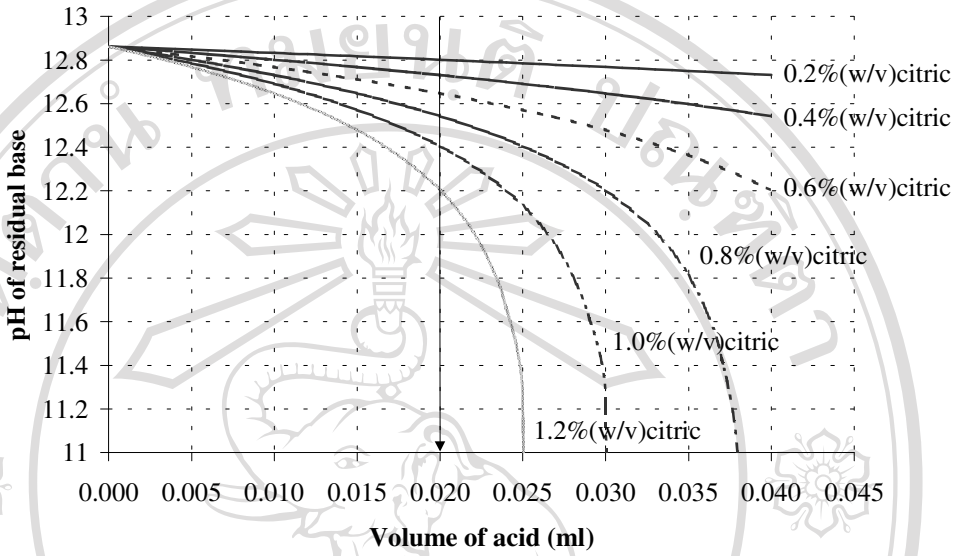


Figure 3.1 Acid-base titration curve.

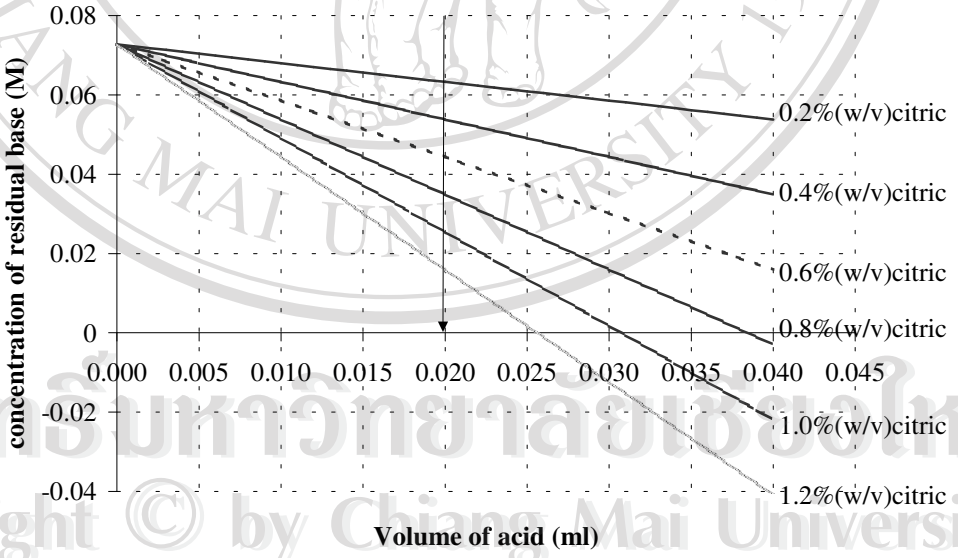
A preliminary condition was obtained based on calculation of the amount of NaOH after complete neutralization with citric acid as illustrated in Figure 3.2b. A 0.12M NaOH (40 µl) was selected as the titrant for determining acidity in concentration range of 0.2 to 1.2 %(w/v) (expressed as citric acid). A volume ratio of NaOH:acid sample solution of 2:1 was selected because it gave a good discrimination among various concentrations of acidity.

A 40 µl NaOH, a 20 µl sample and a 6 µl indicator were sequentially injected into a holding coil and sandwiched by air segments to form a monosegment solution as shown in Figure 3.4. By moving the monosegment forward and backward, the solutions were mixed well by turbulent flow. This mixed solution was then sent to a spectrophotometer and the absorbance of the indicator was monitored at the optimum

wavelength of 608.9 nm (see Figure 3.3). The preliminary condition was summarized as shown in Figure 3.4 and was used to construct a calibration graph.



(a)



(b)

Figure 3.2 Titration graphs of acidity determination to set up preliminary conditions

(a) between the pH of residual base and volume of acid (ml) and

(b) between the concentration of residual base (M) and volume of acid.

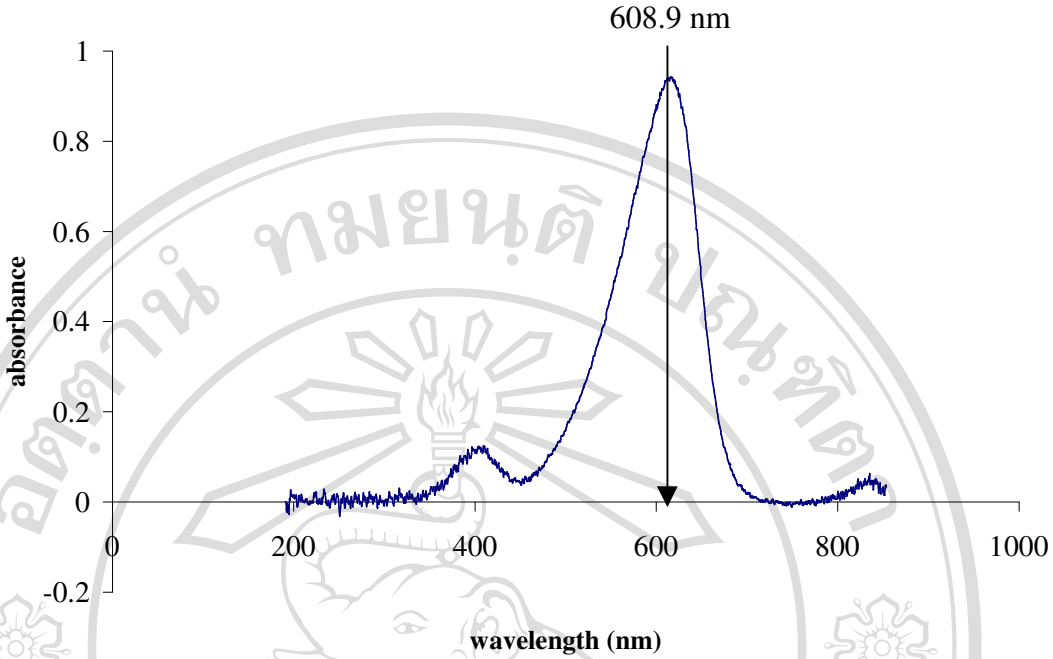
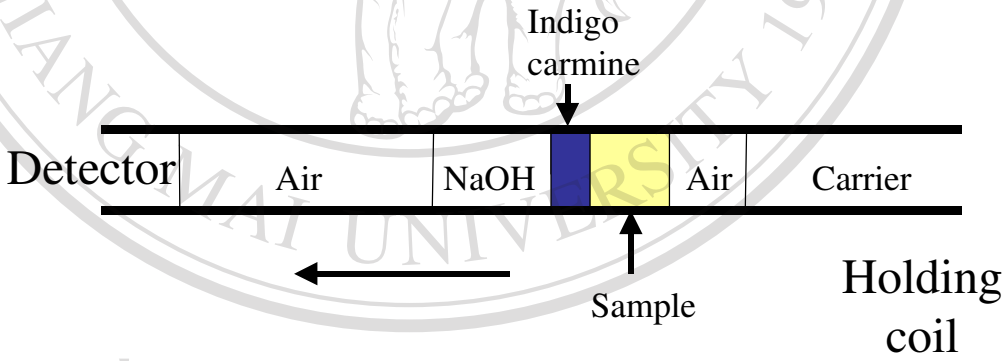


Figure 3.3 Spectrum of indigo carmine indicator.



Volume of zone	200 40 6 20 15	µl
Concentration	NaOH	0.12M
	Indigo carmine	0.10%(w/v)
	Standard citric acid	0.0-1.2%(w/v)
Flow rate	8.33 µl s^{-1} for aspiration and dispensation	
Wavelength	608.9 nm	

Figure 3.4 Summarization of preliminary condition for acidity determination.

Indigo carmine concentration of 0.10%(w/v) was preliminarily selected. The aspiration and dispensation flow rate of 8.33 µl s^{-1} was used to increase dispersion of the solution zone. Using the condition as shown in Figure 3.4, the signal profiles and a calibration graph were obtained as illustrated in Figure 3.5 and 3.6

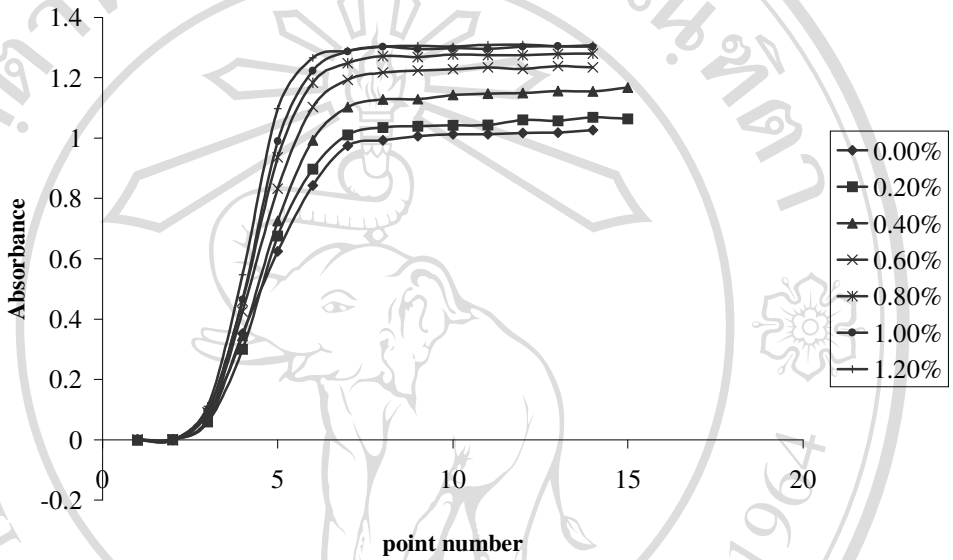


Figure 3.5 Signal profile of various acidity values using the preliminary condition.

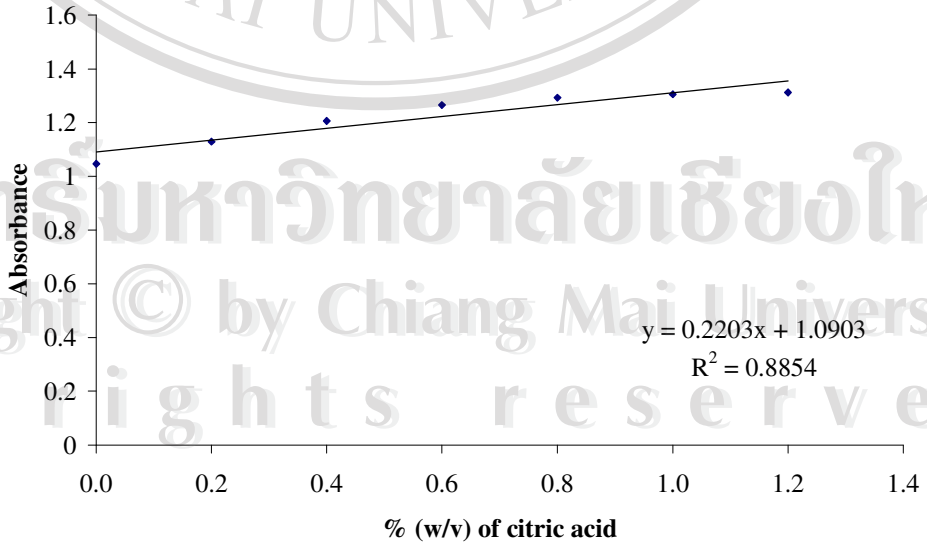


Figure 3.6 Calibration graph obtained using the preliminary condition.

In Figure 3.5, the absorbance was recorded at the rate of 2 Hz (point/sec) i.e. one absorbance value was detected and recorded every half a second. The number of absorbance data point depends on the dispensation flow rate and the volume of the solution. The lower flow rate and higher volume of solution provide were data points. Approximately 16 absorbance points were recorded when using flow rate of 8.33 µl s^{-1} and volume of detected zone of 60 µl. The results in the Figure 3.5 show that, the absorbance was increased at the beginning of the profile. This is because of the zone of solution is moving into the detection point following the water carrier solution. Steady state signal indicates that the solution zones were well mixed and combined to form a monosegment and therefore, a point number was stabilized. Any points of the steady state signal can be selected for constructing a calibration graph. In these profiles, point number 10 was selected because it is in a middle of the zone.

A calibration graph in the Figure 3.6 indicates that the preliminary condition can be used for determination of acidity by SIA-LOV method. However, the slope of the calibration is quite low so some conditions were studied and optimized to improve sensitivity of this technique.

3.2 Determination of acidity by SIA-LOV method

3.2.1 Effect of sugar concentration

Fruit juices contain variety of organic matters. The most common one is sugar which either added or naturally present in juices. Effect of the concentration of sugar

on the determination of acidity by SIA-LOV was studied in the following 2 experiments:

(1) Water and 2%(w/v) sugar were used as reference solutions. The results were compared as shown in Table 3.1 and Figure 3.7, see section 2.4.3.1 (1).

Table 3.1 The absorbance of 0-5%(w/v) sugar solution with water and 2%(w/v) sugar as reference solution.

%(w/v) sugar	**Absorbance* with reference solution**	
	water	**2%(w/v) sugar**
0% (water)	0.004	0.015
1%	-0.001	0.009
2%	-0.010	0.003
3%	-0.021	-0.002
4%	-0.020	-0.004
5%	-0.020	-0.011

*mean of triplicate injections

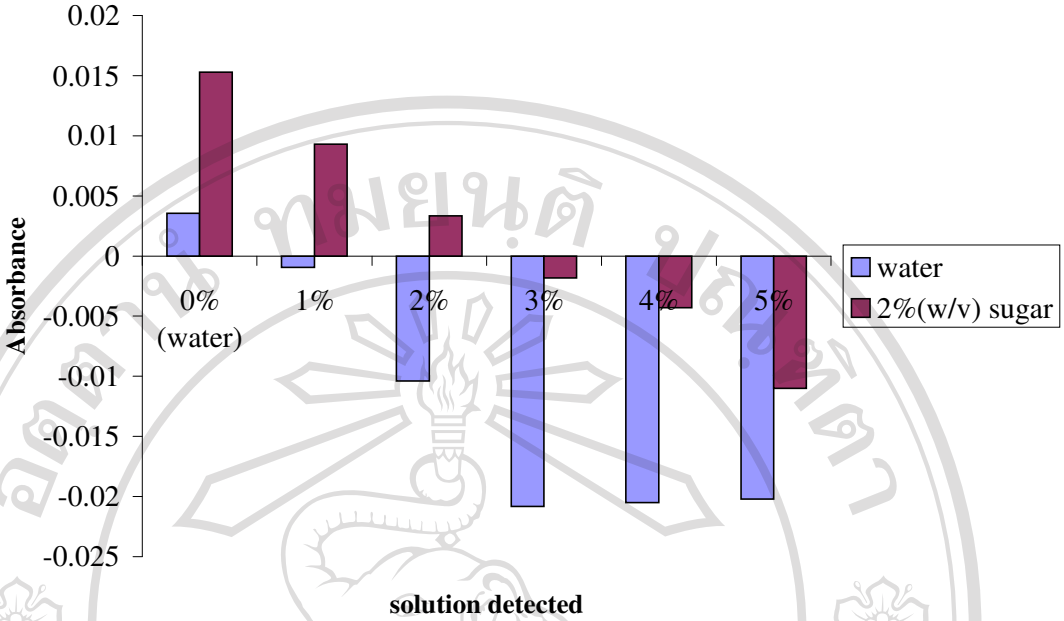


Figure 3.7 Effect of concentration of sugar in solution.

It was found that when using either water or 2%(w/v) sugar as a reference solution, the absorbance was decreased if the concentration of sugar was increased. However, at 3%(w/v) sugar or at higher concentration in water, the absorbance was constant. When 2%(w/v) sugar was used as a reference, the absorbance values seemed to be more correct as compared to when water was used. Normally, the label amounts of sugar in sample are approximately 10%(w/v) . Therefore, the series of standard citric acid was prepared in 10%(w/v) sugar solution.

(2) Two calibration graphs constructed from standard solution with and without 10%(w/v) were compared, see section 2.4.3.1 (2). The results are shown in Figure 3.8 and Table 3.2 and the calibration graphs are shown in Figure 3.9

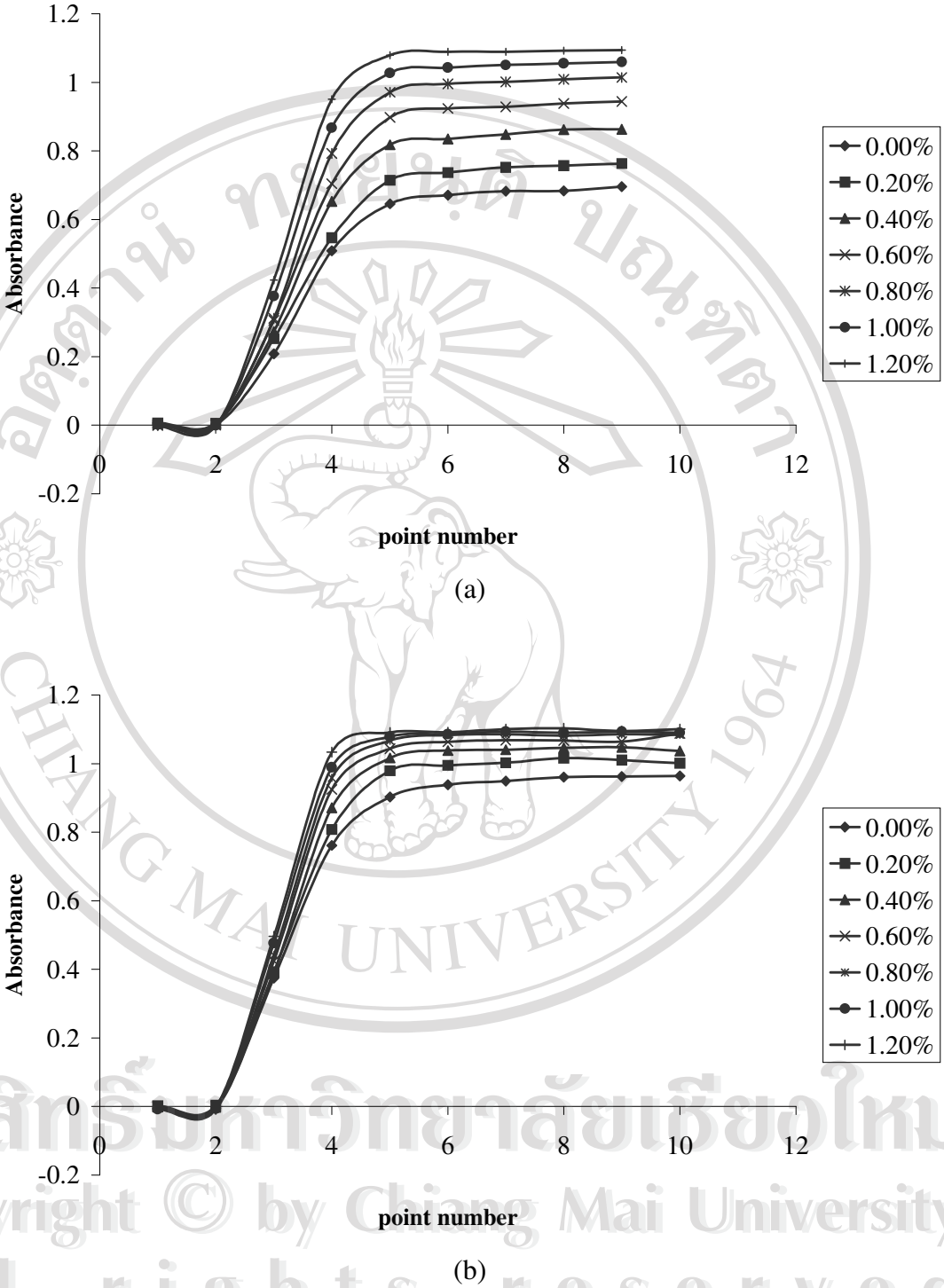


Figure 3.8 Peak profiles of 0.0-1.2% (w/v) citric acid in (a) water and (b) 10% (w/v) sugar solution.

Table 3.2 Effect of the sugar concentration.

Citric acid concentration (%w/v)	Absorbance* in medium	
	water	**10%(w/v) sugar**
0.0	0.683	0.95
0.2	0.752	1.003
0.4	0.848	1.041
0.6	0.929	1.069
0.8	1.002	1.086
1.0	1.051	1.093
1.2	1.09	1.101
Equation	y = 0.3520x + 0.6965	y = 0.1212x + 0.9762
R^2	0.9831	0.8921

* mean of triplicate injections

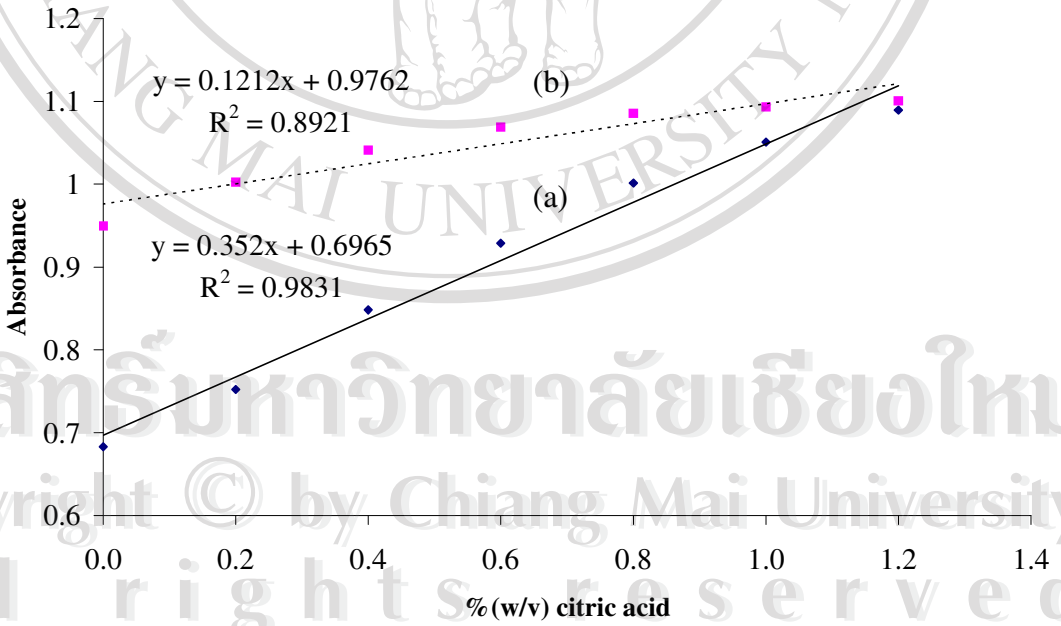


Figure 3.9 A calibration graph of 0.0-1.2%(w/v) citric acid (a) in water and (b) in 10%(w/v) sugar solution.

The results show that sensitivity was decreased when the concentration of sugar in citric acid solution was increased. At low concentration of citric acid, absorbance of the solution with sugar was much higher than without sugar. This may be due to light scattering or light refraction of high density and viscosity sugar solution.

The interference from sugar in sample can be overcome by adding sugar into the standard solution to adjust density and viscosity of the standard solution to be similar to those of sample solution.

3.2.2 Concentration of indigo carmine indicator

The effect of concentration of indigo carmine indicator on the sensitivity of the method was studied. The results are shown in Figure 3.10 and Table 3.3. The slope of calibration graphs are plotted with concentration of indigo carmine indicator, as shown in Figure 3.11 and 3.12.

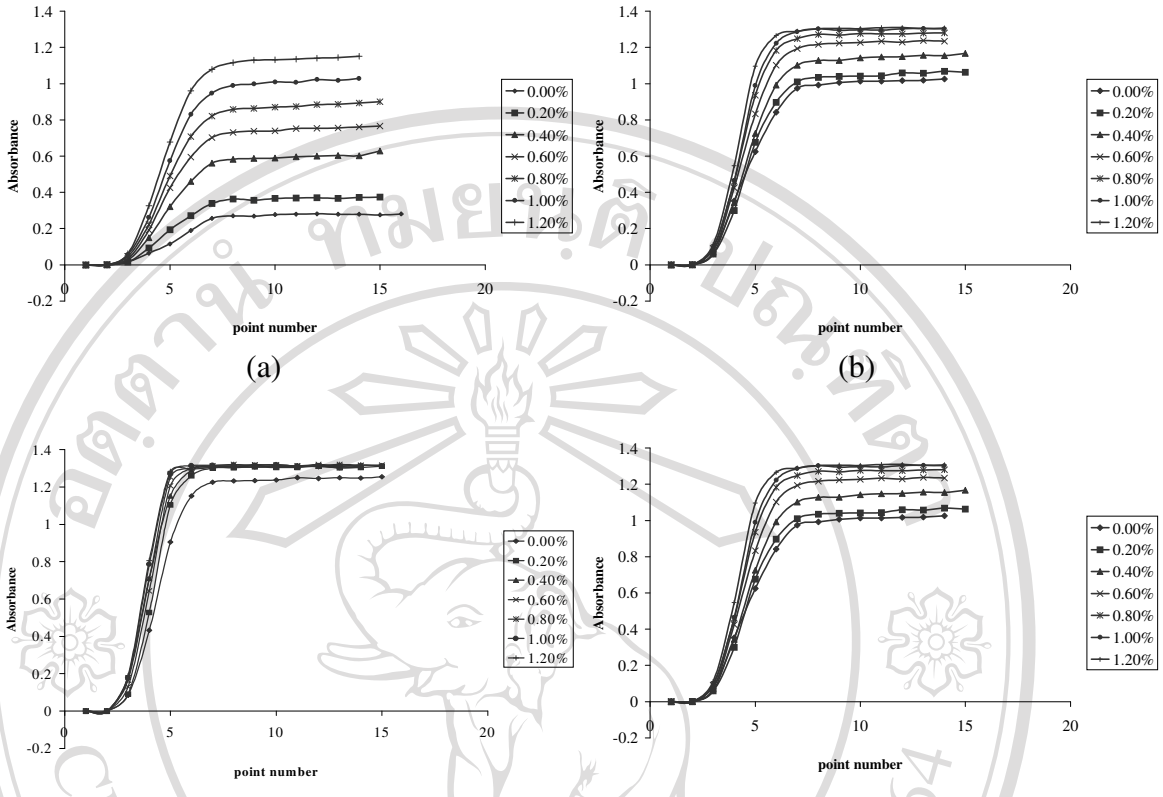


Figure 3.10 Peaks profile of 0.0-1.2%(w/v) citric acid using concentrations of indigo carmine indicator as follows: (a) 0.05%(w/v), (b) 0.10%(w/v), (c) 0.20% (w/v) and (d) 0.40%(w/v).

Table 3.3 Effect of concentration of indigo carmine indicator.

Citric acid concentration (%w/v)	Absorbance* with indico carmine concentration (%,w/v)			
	0.05	**0.10**	**0.20**	**0.40**
0.0	0.277	1.013	1.237	1.324
0.2	0.366	1.042	1.304	1.329
0.4	0.589	1.143	1.308	1.328
0.6	0.739	1.228	1.315	1.33
0.8	0.871	1.277	1.32	1.336
1.0	1.009	1.297	1.319	1.32
1.2	1.131	1.304	1.309	1.318
Equation	y = 0.7376x + 0.2692	y = 0.2709x + 1.0238	y = 0.0460s + 1.2740	y = -0.0054x + 1.3298
R^2	0.9925	0.9261	0.466	0.1436

*mean of triplicate injections

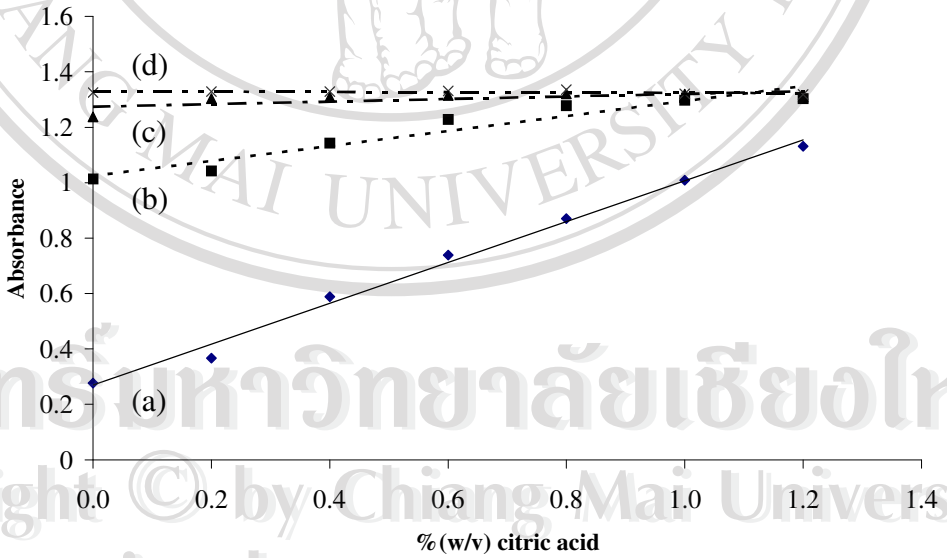


Figure 3.11 Calibration graphs obtained using different concentrations of indigo carmine indicator: (a) 0.05%(w/v), (b) 0.10%(w/v), (c) 0.20%(w/v) and (d) 0.40%(w/v).

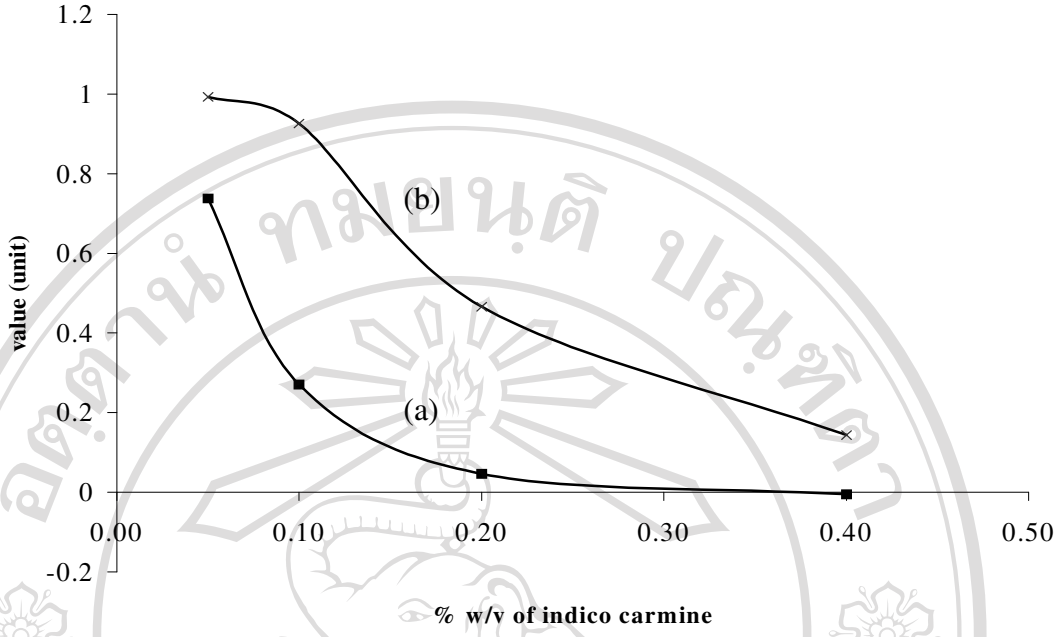


Figure 3.12 Effect of concentration of indigo carmine indicator on (a) slope and (b) R^2 of calibration graph.

From the results, it was found that the slope and R^2 of the calibration graph were decreased when the concentration of indigo carmine was increased. So the 0.05%(w/v) indigo carmine indicator was selected because it gave high slope and R^2 values.

3.2.3 Effect of dispensation flow rate

The effect of dispensation flow rate of the stack zone on sensitivity was studied. The results are shown in Figure 3.13 and 3.14.

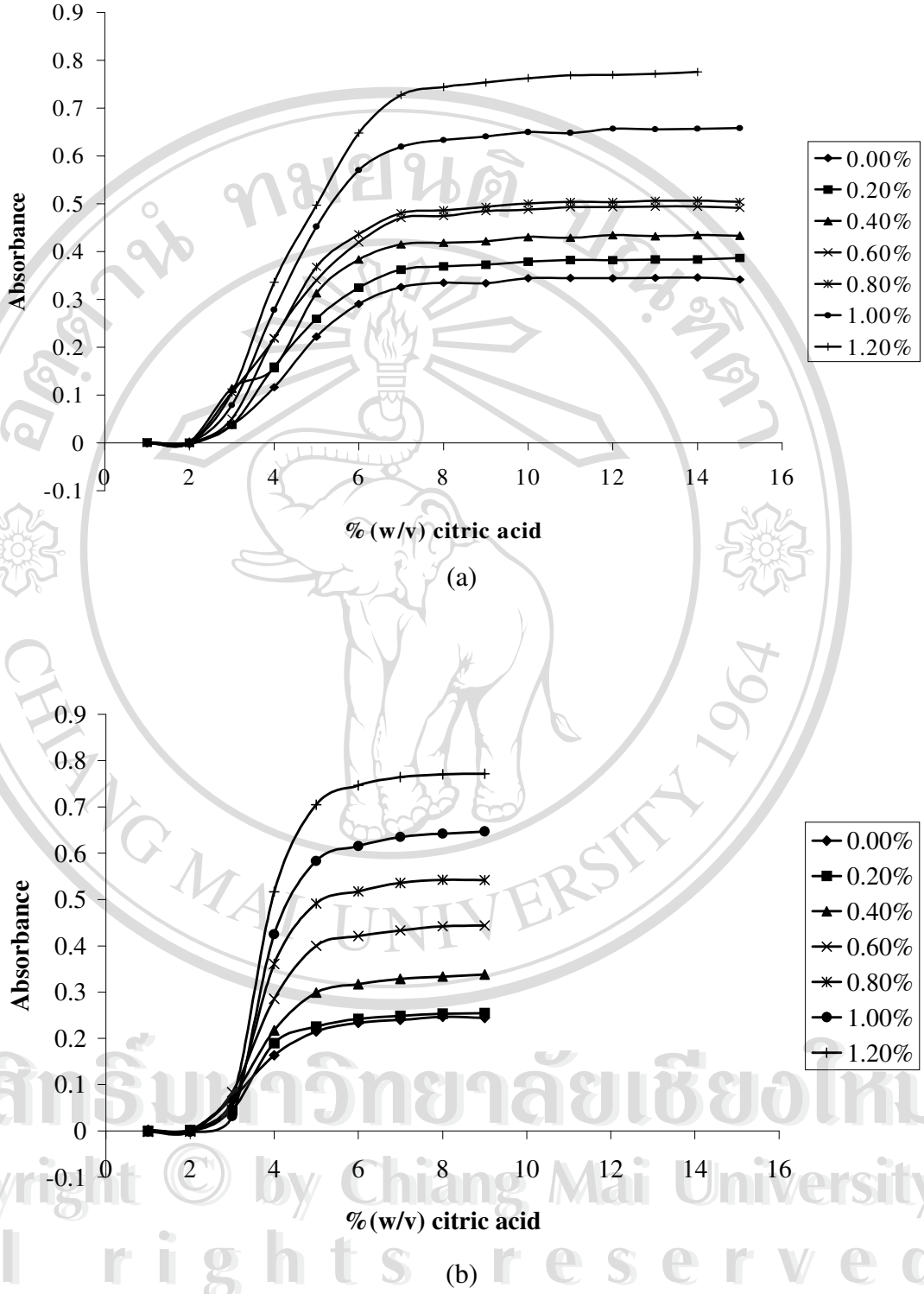


Figure 3.13 Peak profiles of 0.0-1.2%(w/v) citric acid using dispensation flow rate of: (a) 8.33 µl s^{-1} and (b) 16.66 µl s^{-1}.

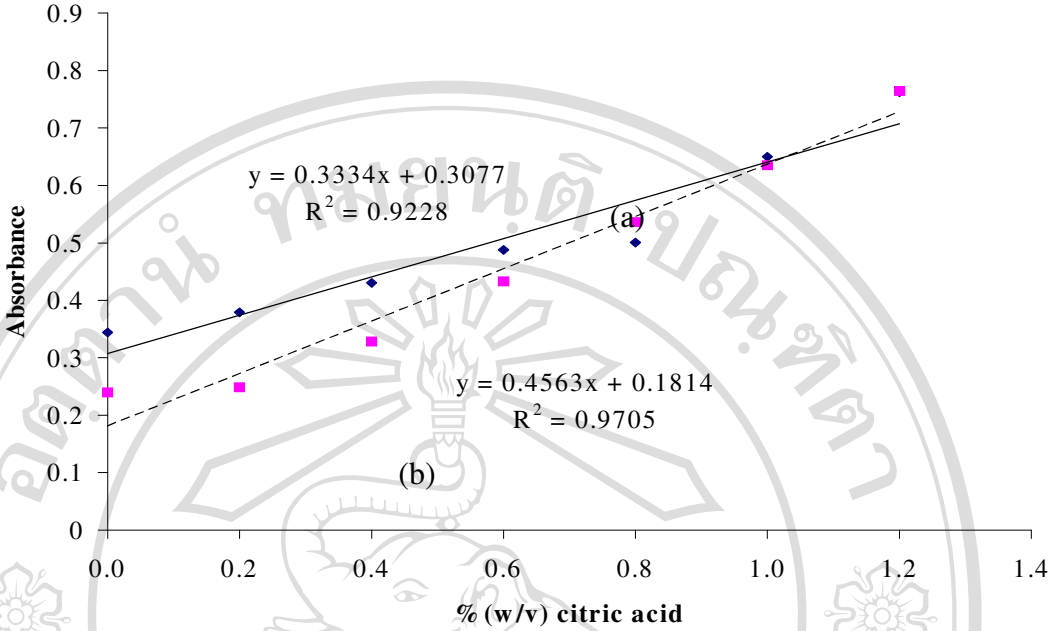


Figure 3.14 Calibration graphs obtained using different dispensation flow rates as follows; (a) 8.33 µl s^{-1} and (b) 16.66 µl s^{-1}.

From the results, it was found that, the number of data points of signal was decreased when the dispensation flow was increased. The number of data points collected at the flow rate of 16.66 µl s^{-1} are enough for signal evaluation, but when the dispensation flow rate is higher than these, the number of data points are insufficient. This is because the number of data points collected depends on the sampling rate and flow rate. A middle point at the steady state signal was selected from each condition. Point number 10 was selected when using dispensation flow rate of 8.33 µl s^{-1} and point number 7 was selected when using flow rate of 16.66 µl s^{-1}.

The flow rate of 16.66 µl s^{-1} was selected because it gave higher sensitivity than the flow rate of 8.33 µl s^{-1}. Moreover, the analysis time was shorten and the sample through-puts of the method was increased to 30 samples/h.

3.2.4 Summary of the selected conditions

The selected conditions for the SIA-LOV determination of acidity in the range of 0.0-1.2%(w/v) as citric acid are shown in Table 3.4.

Table 3.4 Conditions for determination 0.0-1.2%(w/v) citric acid.

Parameter	Condition
Reagent solution	40 µl of 0.12M sodium hydroxide 6 µl of 0.05%(w/v) indigo carmine indicator
Sample volume	20 µl
Volume of air	15 µl for the end of zone and 200 µl for the beginning of zone (see Figure 2.2)
Blank solution	10%(w/v) of sugar solution
Flow rate	8.33 µl s^{-1} for aspiration 16.66 µl s^{-1} for dispensation to detector
Volume of detection	80 µl
Wavelength	608.9 nm

3.2.5 Determination of acidity in fruit juice samples

3.2.5.1 Calibration graph

The experiment was carried out as described in the section 2.4.4. The peak profile and a calibration graph obtained in the range of 0.0-1.2%(w/v) citric acid are shown in Figure 3.15, Table 3.5 and Figure 3.16 respectively.

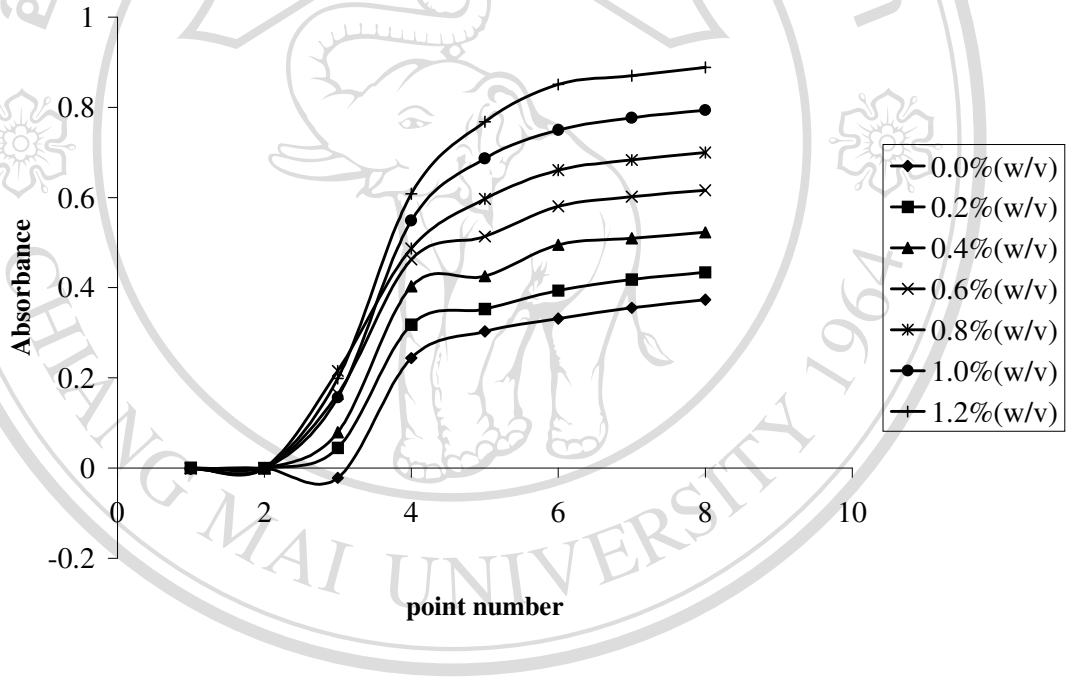


Figure 3.15 Peak profiles of 0.0-1.2%(w/v) citric acid.

Table 3.5 Calibration data of 0.0-1.2%(w/v) citric acid.

Citric acid concentration (%w/v)	Absorbance*
0.0	0.356
0.2	0.418
0.4	0.510
0.6	0.601
0.8	0.683
1.0	0.776
1.2	0.870

* mean of triplicate injections

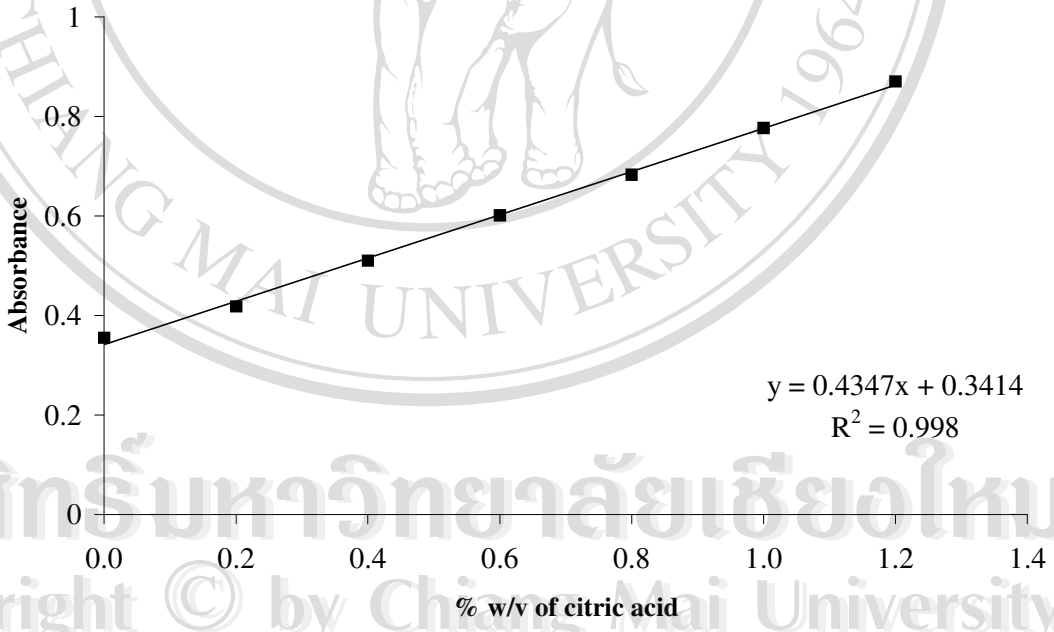


Figure 3.16 A calibration graph method for determination of acidity obtained from SIA-LOV.

The calibration graph in Figure 3.16 can be used to determine acidity of fruit juice samples.

3.2.5.2 Accuracy and application of the method

Accuracy of the proposed method was determined by comparing results from SIA-LOV with those from a standard titrimetric method. Comparative analyses of the same samples were carried out on the same day and the results are shown in Table 3.6, Figure 3.17 and Figure 3.18.

Table 3.6 Comparison of % acidity found (expressed as citric acid) by SIA-LOV and standard titration method.

Sample code	Type of fruit juice	Citric acid found (%(w/v)[a]		Relative error (%)[b]
		Titration	SIA-LOV	
A	Grape	0.226	0.389	72.07
B	Grape	0.286	0.376	31.74
C	Lychee	0.297	0.330	11.08
D	Grape	0.320	0.959	199.73
E	Lime	0.373	0.392	5.24
F	Lychee and pineapple	0.412	0.440	6.86
G	Grape	0.412	1.309	217.94
H	Pineapple	0.425	0.433	1.95
I	Lime	0.443	0.468	5.81
J	Apple and pineapple	0.450	0.490	8.92
K	Orange	0.451	0.484	7.26
L	Pineapple	0.496	0.530	6.77
M	Apple	0.518	0.578	11.59
N	Guava	0.610	0.726	19.10
O	Guava	0.678	1.090	60.69
P	Pineapple	0.907	0.935	3.11
Q	Pineapple	1.021	1.081	5.87

[a] mean of triplicate injections

[b] Relative error (%) = $\frac{\text{[SIA-LOV value – titration value] x 100}}{\text{titration value}}$

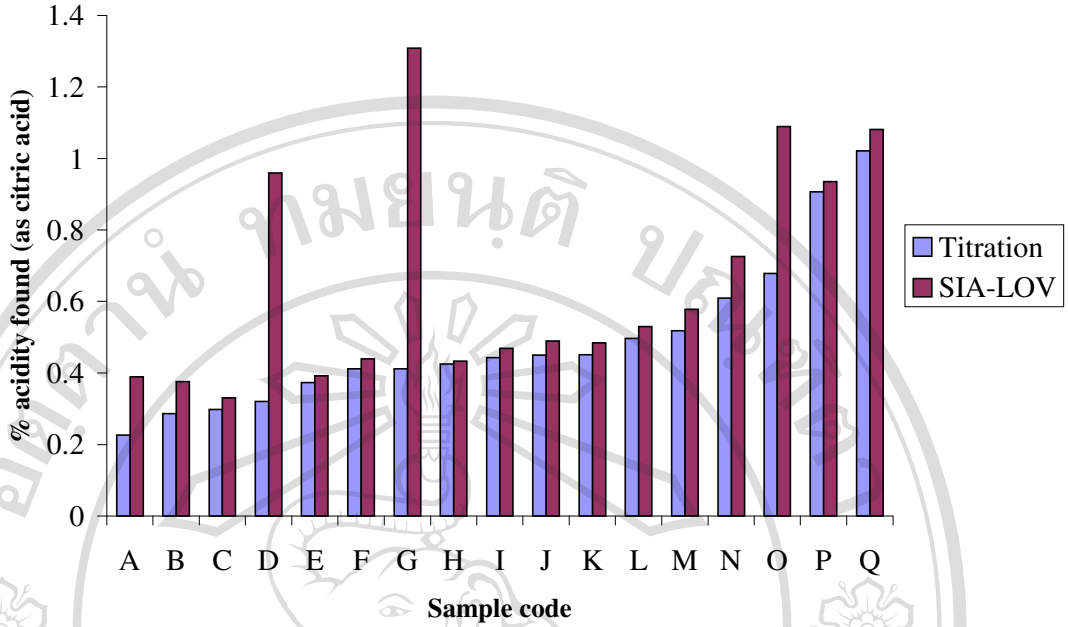


Figure 3.17 Comparison graph of acidity found in fruit juice samples by SIA-LOV and titration method.

From table 3.6 and Figure 3.17, acidity found in fruit juice samples (expressed as citric acid concentration) by using SIA-LOV method agreed well with those found by the standard titration method, except for grape juices that has the color similar to the color of an indicator and the color so interfered the analysis seriously. The correlation of the two methods is shown in Figure 3.18.

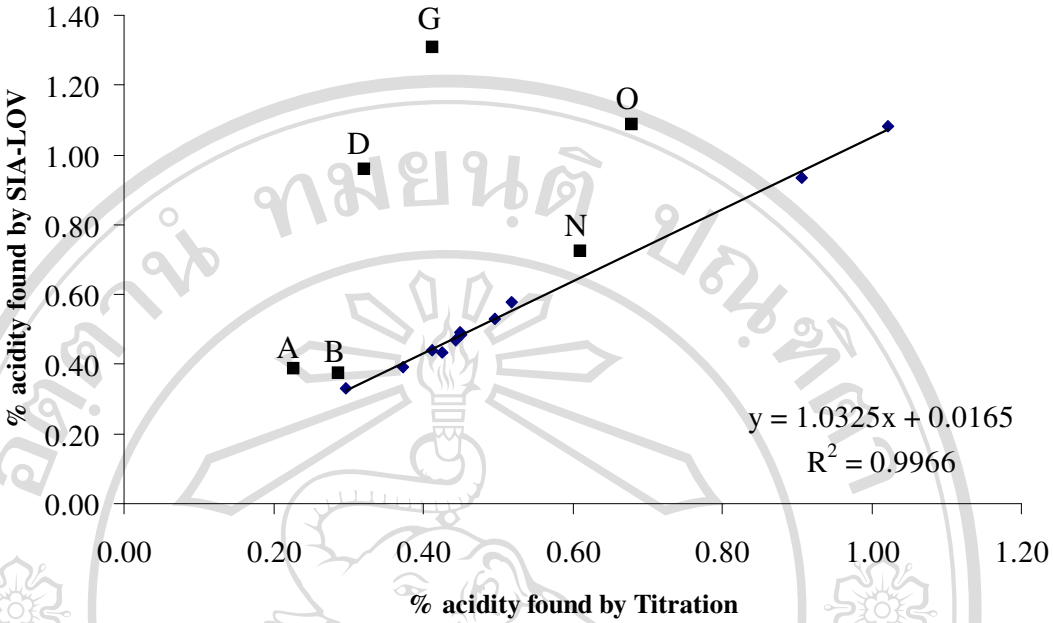


Figure 3.18 Correlation graph of %(w/v) acidity found by using SIA-LOV and titration method.

Slope and intercept of the correlation graphs of the fruit juices, except sample A, B, D, G, N and O are closed to 1 and 0.0165, respectively. This indicates that both methods correlate well. The sample A, B, D and G are grape juices with purple color which interfered the color of indicator. Therefore, the acidity found in grape juices by SIA-LOV method were higher than that by the standard titration method.

Table 3.7 The summarization of t-test value for acidity determination in fruit juice. [34]

Sample code	Mean of acidity found (%,w/v)[a]		SD of themethod		N	S of t [b]	t calculate [c]	t table at 95% confidence (N=4)	NOTE
	Titration	SIA-LOV	Titration	SIA-LOV					
A	0.226	0.389	0.021	0.026	4	0.023	5.738	2.78	significantly different
B	0.286	0.376	0.000	0.021	4	0.015	4.916	2.78	significantly different
C	0.297	0.330	0.000	0.007	4	0.005	5.101	2.78	significantly different
D	0.320	0.959	0.000	0.023	4	0.016	32.708	2.78	significantly different
E	0.373	0.392	0.022	0.034	4	0.029	0.555	2.78	non different
F	0.412	0.440	0.000	0.035	4	0.025	0.939	2.78	non different
G	0.412	1.309	0.000	0.010	4	0.007	99.091	2.78	significantly different
H	0.425	0.433	0.013	0.008	4	0.011	0.636	2.78	non different
I	0.443	0.468	0.006	0.006	4	0.006	3.584	2.78	significantly different
J	0.450	0.490	0.013	0.024	4	0.020	1.666	2.78	non different
K	0.451	0.484	0.005	0.037	4	0.027	0.998	2.78	non different
L	0.496	0.530	0.013	0.025	4	0.020	1.391	2.78	non different
M	0.518	0.578	0.013	0.019	4	0.016	3.011	2.78	significantly different
N	0.610	0.726	0.026	0.008	4	0.019	4.900	2.78	significantly different
O	0.678	1.090	0.026	0.041	4	0.034	9.816	2.78	significantly different
P	0.907	0.935	0.026	0.007	4	0.019	1.194	2.78	non different
Q	1.021	1.081	0.026	0.020	4	0.024	2.078	2.78	non different

[a] mean of triplicates

[b] $s = \left[\frac{(2SD^2_{titration} + 2SD^2_{SIA\text{-}LOV})}{N} \right]^{1/2}$

[c] $t = \frac{(mean_{titration} - mean_{SIA\text{-}LOV}) \times (2/3)^{1/2}}{s}$

Some sample in the Table 3.7 are significantly different from standard titration method. The major reason of this problem is because some samples that contain other organic acids as major contents that can interfere the analysis of citric acid. Examples of these juices are Lychee and Guava juices (C, N and O) which contain ascorbic acid [32] and apple juice (M) which contain malic acid [33].

For the sample I is lime juice. The results are significantly different because the dilution of sample in standard method may affect the determination.

3.2.6 Precision

A solution containing 0.6%(w/v) citric acid and 10%(w/v) sugar was used to study precision of the proposed method. The analysis of 11 replicates was done under the selected condition. The results are shown in Figure 3.19 and Table 3.8.

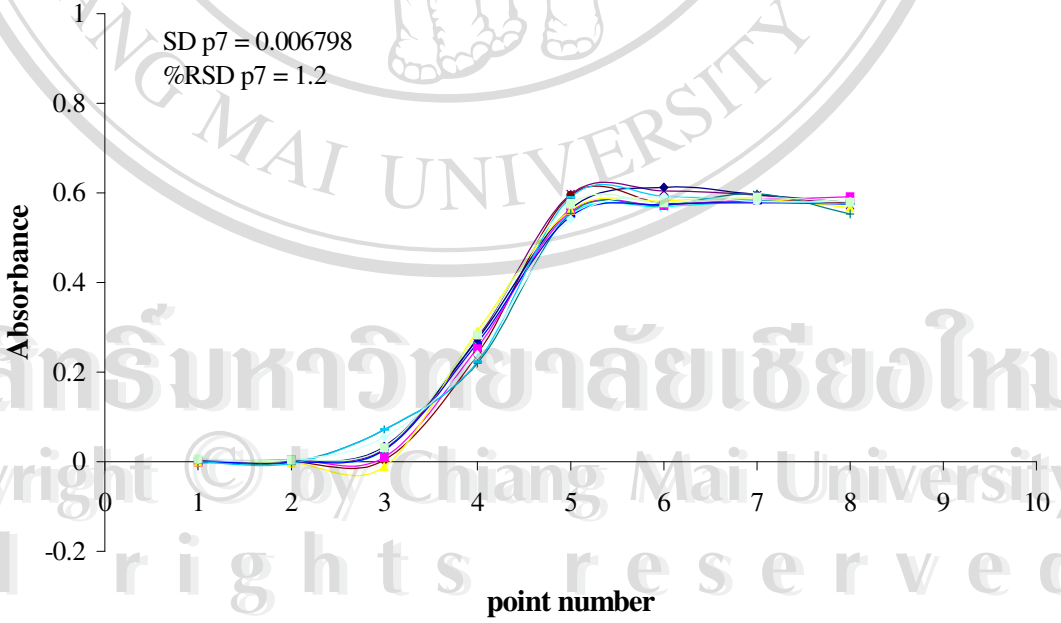


Figure 3.19 Peak profiles of 11 replicates of 0.6%(w/v) citric acid in 10%(w/v) sugar solution.

Table 3.8 Precision of citric acid determination by SIA-LOV method.

Number of injection	Absorbance (point 7)
1	0.583
2	0.595
3	0.583
4	0.596
5	0.585
6	0.585
7	0.597
8	0.578
9	0.582
10	0.580
11	0.590
mean	0.587
SD	0.007
%RSD	1.2

From the results, it was found that, the relative standard deviation (RSD) obtained was 1.2%. This indicates that the proposed system has a good precision.