CHAPTER II

EXPERIMENT

2.1 Instruments, apparatus and chemicals.

2.1.1 SIA-LOV analyzer

FIAlab3000 (FIAlab, Washington, USA) consists of

- Syringe pump (2500 µl)
- Six-port multiposition valve
- Multi-purpose flow cell (lab-on-valve)
- Fiber optic spectrophotometer, OOIBase32
- 2-channels peristaltic pump
- FIAlab 5.0 program

Personal computer (1.70MHz Pentium4®, Intel corporation, Santa Clara, USA)

2.1.2 Chemicals

All chemicals were analytical-reagent grade except when specified.

1. Alizarin yellow indicator: $C_{13}H_9N_3O_5$, Merck
2. Citric acid: $C_6H_8O_7.H_2O$, Carlo Erba
3. Indigo carmine indicator: $C_{16}H_8N_2O_8S_2Na_2$, BDH
4. Phenolphthalein indicator: $C_{20}H_{14}O_4$, Merck
5. Potassium hydrogen phthalate: $C_8H_5O_4K$, Univar
6. Sodium hydroxide: NaOH, lab-scan

7. White cane sugar

2.2 Samples

2.2.1 Samples for acidity determination

Acidity in fruit juice samples were investigated by a standard titration method (see APPENDIX A). Details of the samples are shown in Table 2.1.

Table 2.1 List of fruit juice samples.

Type of fruit juice	Brand	Concentration of fruit juice (%v/v)	Sample code
Grape juice	Deedo	25%	A
Grape juice	Golden Pan	25%	B
Lychee juice	Chabaa	30%	C
Grape juice	Unif Daily C	40%	D
Lime juice	Golden Pan	25%	E
Lychee juice and pineapple juice	Tipco Cool	40%	F
Grape juice	Chabaa	30%	G
Pineapple juice	Golden Pan	25%	H
Lime juice	Deedo	15%	I
Apple juice and pineapple juice	Tipco Cool	40%	J
Orange juice	Golden Pan	25%	K
Pineapple juice	Deedo	25%	L
Apple juice	Malee	25%	M

Table 2.1 (cont.)

Type of fruit juice	Brand	Concentration of fruit juice (%v/v)	Sample code
Guava juice	Unif Daily C	40%	N
Guava juice	UFC	30%	O
Pineapple juice	UFC	100%	P
Pineapple juice	Tipco	100%	Q

2.2.2 Samples preparation

Some fruit juice samples that contain some solid particles such as fruit pulps were centrifuged and filtered prior to use. The clear sample solutions were kept in the refrigerator to analyze within one week.

2.3 Preparation of standard solutions and reagents

2.3.1 Stock standard solution of citric acid, 10.0%(w/v)

Standard solution of 10.0%(w/v) citric acid was prepared by dissolving 27.3420 g of citric acid ($C_6H_8O_7.H_2O$ M.W.=210.14) in 250 ml of deionized water. The working solutions were prepared by diluting the stock solution with deionized water.

2.3.2 Stock standard solution of citric acid, 10.0%(w/v) in 10%(w/v) sugar solution

This standard solution was prepared by dissolving 27.3420 g of citric acid in 250 ml of 10% w/v sugar (sucrose) solution. The working solutions were prepared by diluting the stock solution with 10%(w/v) sugar solution.

2.3.3 Stock standard solution of sodium hydroxide, 0.2M

A solution was prepared by dissolving 2.00 g of sodium hydroxide in 250 ml deionized water and standardized with 0.10 M potassium hydrogen phthalate using phenolphthalein as an indicator. The working solutions were prepared by diluting the stock solution with deionized water. It was standardized with 0.10 M potassium hydrogen phthalate if the exact concentration was required.

2.3.4 Sugar solution, 2%w/v

Exactly 2.00 g of white sugar (sucrose) was dissolved in 100 ml deionized water. Other concentrations were prepared in the same manner.

2.3.5 Indigo carmine indicator solution, 0.1%w/v

A 0.10 g of indigo carmine indicator was dissolved in 100 ml deionized water. Other concentrations were prepared in the same manner.

2.4 Determination of acidity by sequential injection with lab-on-valve method

2.4.1 SIA-LOV system

A schematic diagram of the sequential injection with lab-on-valve system is shown in Figure 2.1. All tubing were 0.6 mm i.d. polytetrafluoroethylene (PTFE). The length of the holding coil (HC) was 50 cm. Multiposition valve used in this system was a six-port valve. The center port was connected to the syringe pump via the holding coil. Port number two (#2) was a detection port where a multi-purpose flow cell was integrated. Port number five (#5) was for sample loading which carried out through a peristaltic pump. The peristaltic pump helped to reduce the operation sequence and analysis time. Other ports (1, 3, 4 and 6) were for loading of other reagents, depending on the system design.

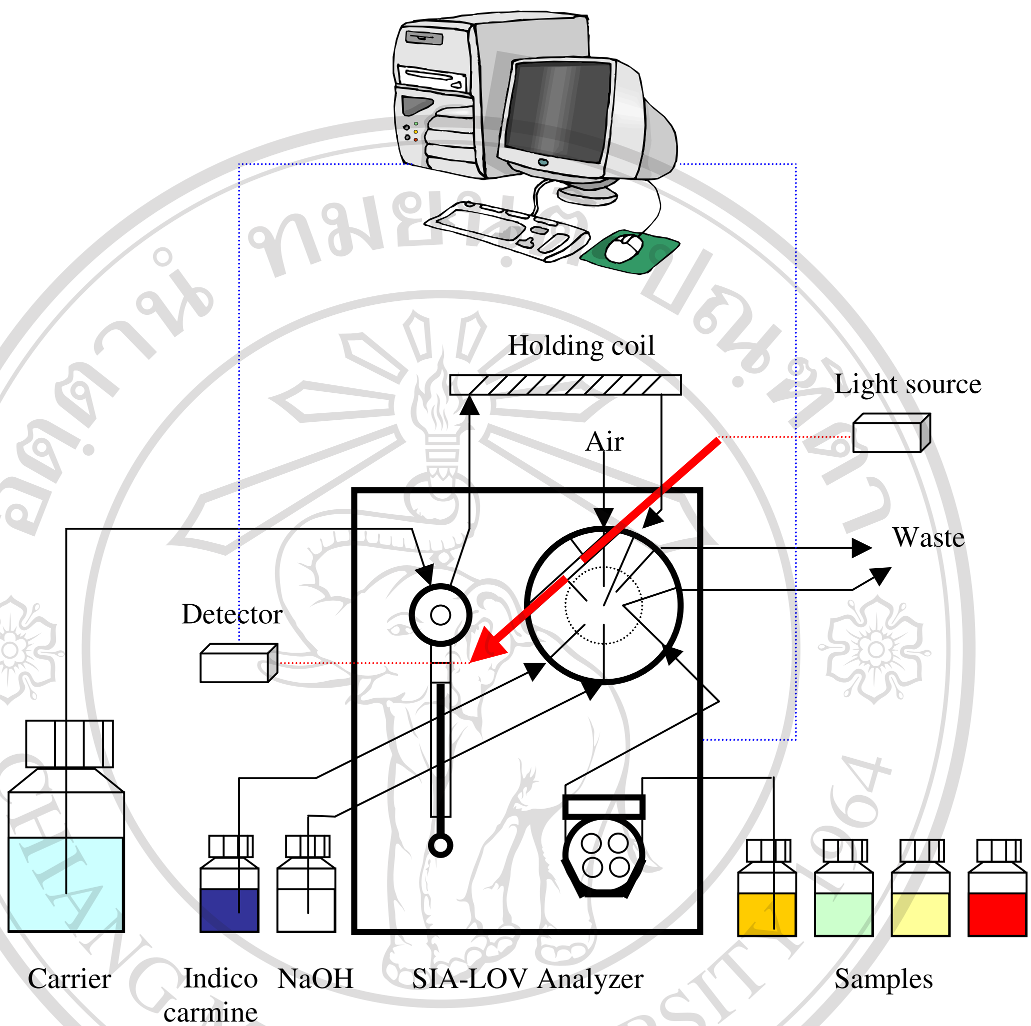

Figure 2.1 A schematic diagram of the SIA-LOV system for acidity determination.

2.4.2 Procedure

Figure 2.1 illustrates a diagram of various components of the computer controlled SIA-LOV system and a manifold setup for the determination of acidity. The operation steps were controlled by a computer program (FIAlab 5.0, see APPENDIX B.1) and are summarized as follows;

1. The carrier, H_2O, was drawn passing all the lines to clean the tube and to remove air from the system. The sample or standard citric acid solutions and reagents were drawn to fill up their lines.

2. A 100 µl of water was propelled into multi-purpose flow cell at the flow rate of 8.33 µl s^{-1} . The reference absorbance of the carrier solution at this condition was detected and memorized. .

3. Air, sample, indigo carmine indicator, NaOH and air were drawn into a holding coil at a flow rate of 8.33 µl s^{-1} through port 1, 5, 3, 4 and 1 respectively, (see Figure 2.2). They were dawn in the following order; an appropriate volume of air segment, 0.0-1.2%(w/v) standard solution or samples, 0.1%(w/v) indigo carmine solution, 0.12M sodium hydroxide solution and followed by another air segment.

4. With the same flow rate as mentioned above, the last air segment was removed through port 6.

5. Finally, the stack zone was propelled at the flow rate of 16.66 µl s^{-1} through a multi-purpose flow cell via port 2. The change in color intensity of the indicator was detected at 608.9 nm.

In all experiments, the measurement cycle was repeated three times for each concentration. The maximum absorbance values were plotted against concentrations of citric acid and printed out by Microsoft ® Excel program.

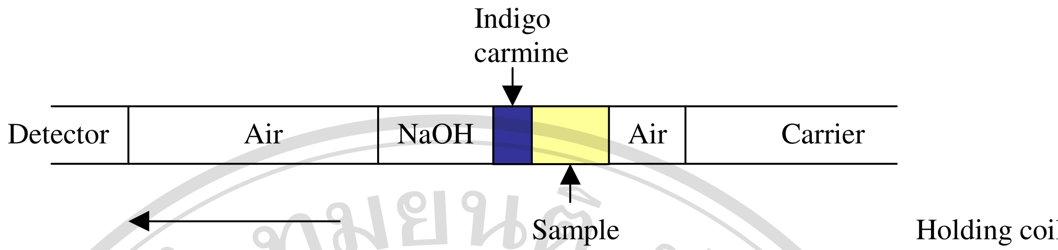

Figure 2.2 The sequence of operation of SIA-LOV.

2.4.3 Optimization of the SIA-LOV system

Preliminary condition was set as shown in Table 2.2

Table 2.2 Preliminary condition for acidity determination.

Parameter	Conditions
Aspiration volume of air	15 µl
Aspiration volume of standard citric acid solution	20 µl
Aspiration volume of indigo carmine indicator	6 µl
Aspiration volume of sodium hydroxide solution	40 µl
Aspiration volume of air	200 µl
Dispensation volume of air	210 µl
Pump-flow rate	8.33 µl s^{-1}
Wavelength	608.9 nm

This method used air segments to improve mixing efficiency and to minimize dispersion so that the sensitivity could be increased.

2.4.3.1 Effect of sugar solution concentration

Two procedures for investigation of the effect of sugar concentration were performed using the system in Figure 2.1 and 2.2 (see FIAlab 5.0 program in APPENDIX B.1).

(1) The new sequence of the experiment was set up as shown in Figure 2.3 using FIAlab 5.0 program (see APPENDIX B.2). First, water carrier solution was used to scan for reference absorbance. Then, absorbances from a series of sugar solutions (1-5%(w/v)) were detected. After that, the reference solution was changed to 2%(w/v) sugar solution and absorbances of the same series of sugar solutions were determined. The absorbance of each peak of both experiments were compared.

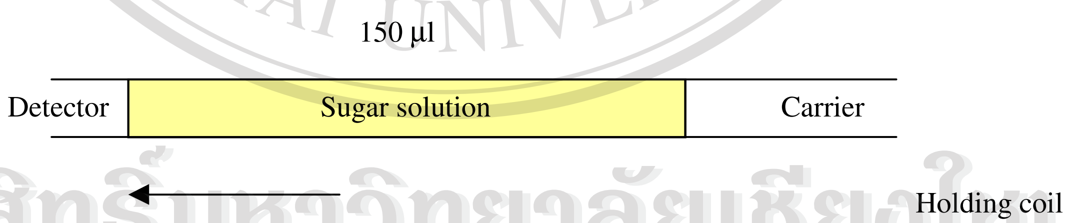

Figure 2.3 The sequence of operation for the study of the effect of sugar concentration.

(2) A blank and a series of standard citric acid solutions in water (0.0-1.2%(w/v)) were injected into the system using the condition that described in Table 2.2. A standard citric acid for the next analysis run was filled into the tube by a peristaltic pump while the current analysis was operating to decrease the operation time.

The same concentration series of standard citric acid solutions but prepared in 10%(w/v) sugar were injected with the same experiment condition as previously mentioned. Two calibration graphs were constructed from peak heights (maximum absorbances) versus concentrations of citric acid in the range of 0.0-1.2% (w/v).

2.4.3.2 Effect of concentration of indigo carmine indicator

The system and the sequence of operation in Figure 2.1 and Table 2.2 were used. A series of indigo carmine solutions at 0.05, 0.1, 0.2, and 0.4%(w/v) were studied for the determination of 0.0-1.2%(w/v) standard citric acid in 2%(w/v) sugar solutions. The optimum concentration of indigo carmine was the one that offered the calibration graph with the highest slope, which indicates the best sensitivity.

2.4.3.3 Effect of dispensation flow rate

The dispensation flow rate of the system at 8.33 and 16.66 $\mu l\ s^{-1}$ was studied for the determination of 0.0-1.2%(w/v) standard citric acid in 10%(w/v) sugar solutions. The optimum flow rate was the one that offered the calibration graph with the highest slope, which indicates the best sensitivity. The system and the sequence of operation in Figure 2.1 and Table 2.2 were used, except, the indigo carmine concentration of was changed to the optimum concentration of 0.05%(w/v).

2.4.4 Determination of acidity in fruit juice samples

The acidity of fruit juice samples was determined using the optimum condition. The absorbance peak heights of samples were used to determine the acidity values based on the calibration graph that constructed from 0.0-1.2%(w/v) standard citric acid in 10%(w/v) sugar solutions. The results were compared to those obtained from AOAC titrimetric method (see APPENDIX A).

2.4.5 Precision

Using the same condition as in 2.4.4, precision of the system was determined from 11 replicated analysis of 0.6%(w/v) standard citric acid in 10%(w/v) sugar solution.