APPENDICES

APPEXDIX A

p-Nitrophenol phosphate stock solution, 0.5 M

Stock standard p-nitrophenyl phosphate solution 0.5 M can be kept at -20°C for 3 days without the lost in sensitivity.

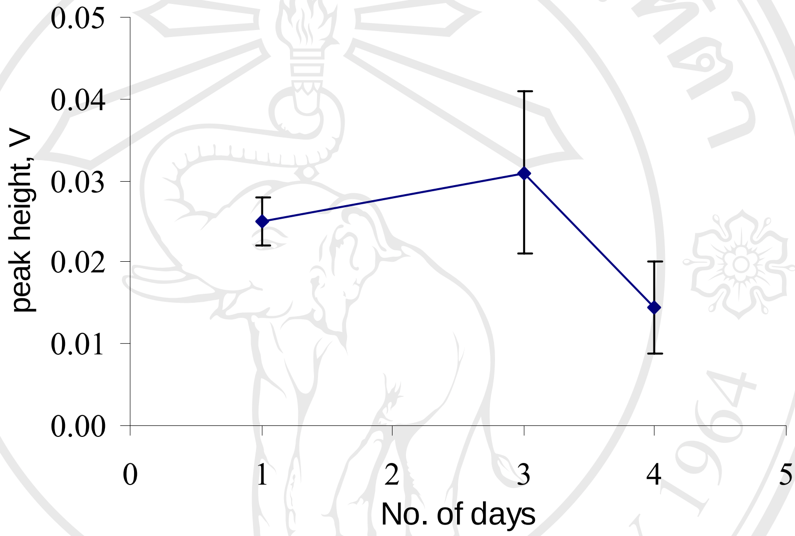
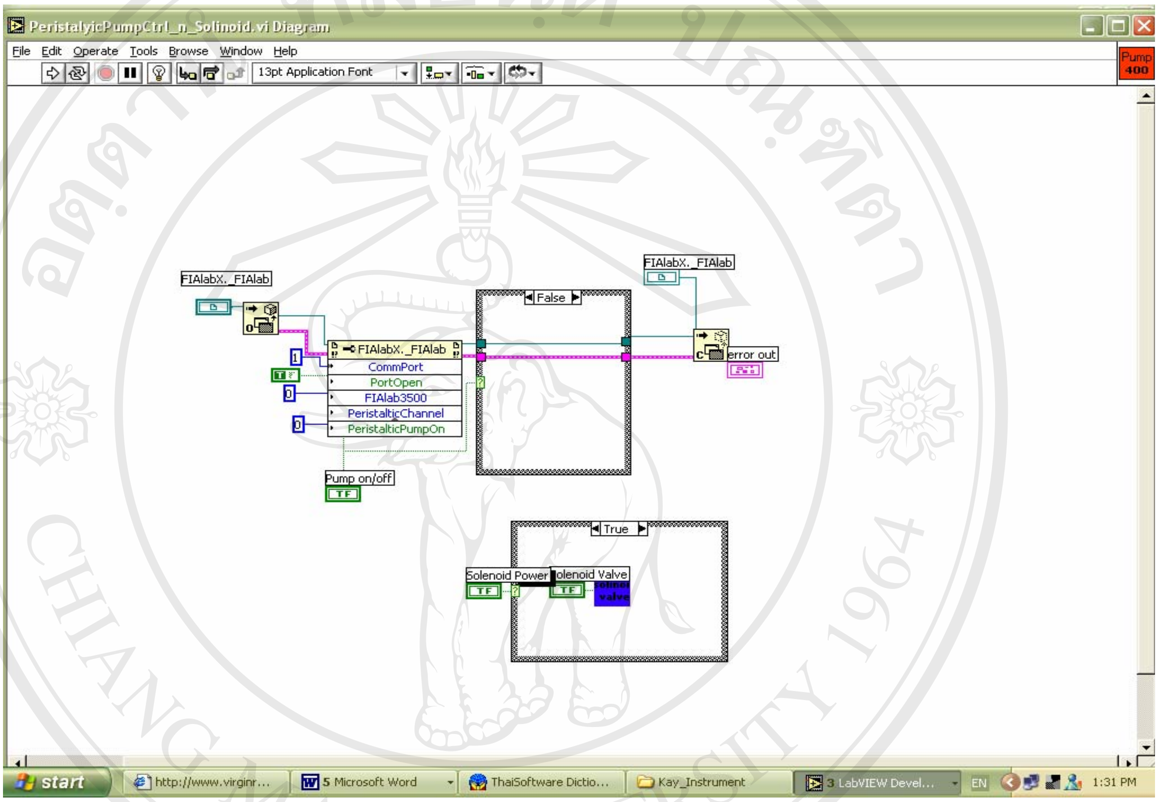


Figure A1 Stability of pNPP stock solution stored at -20°C

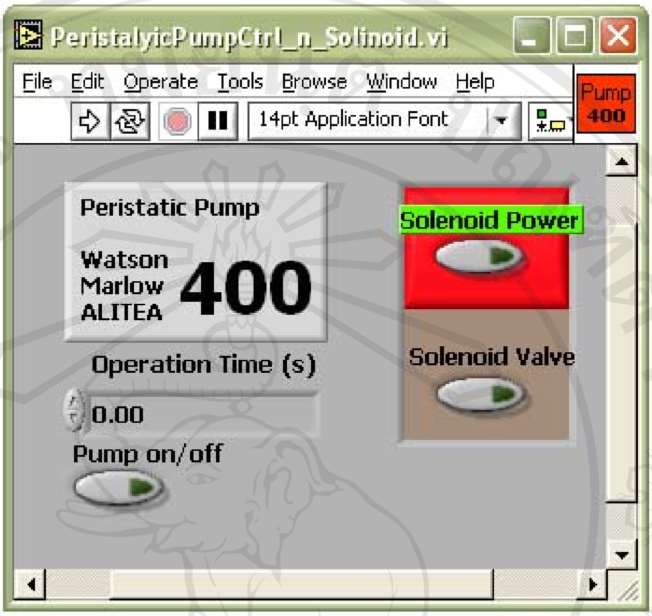
APPEXDIX B

1. LabView® computer program for controlling the BI system

1.1 Diagram of LabView® computer program for controlling the BI system



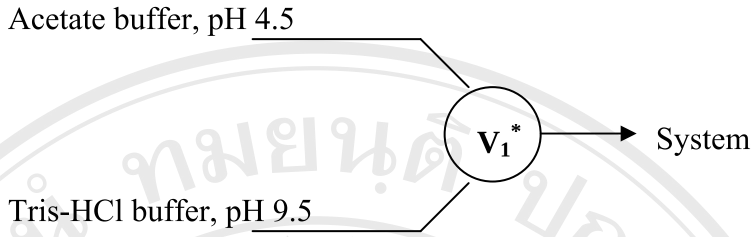
1.2 Display of LabView® computer program for controlling the BI system



	Bead retention		Bead discarding
	Bead loading	Bead packing	
Pump*	On	On	On
Operation time (s)	10	100	100
Solenoid power	On	On	On
Solenoid valve	Off	Off	On

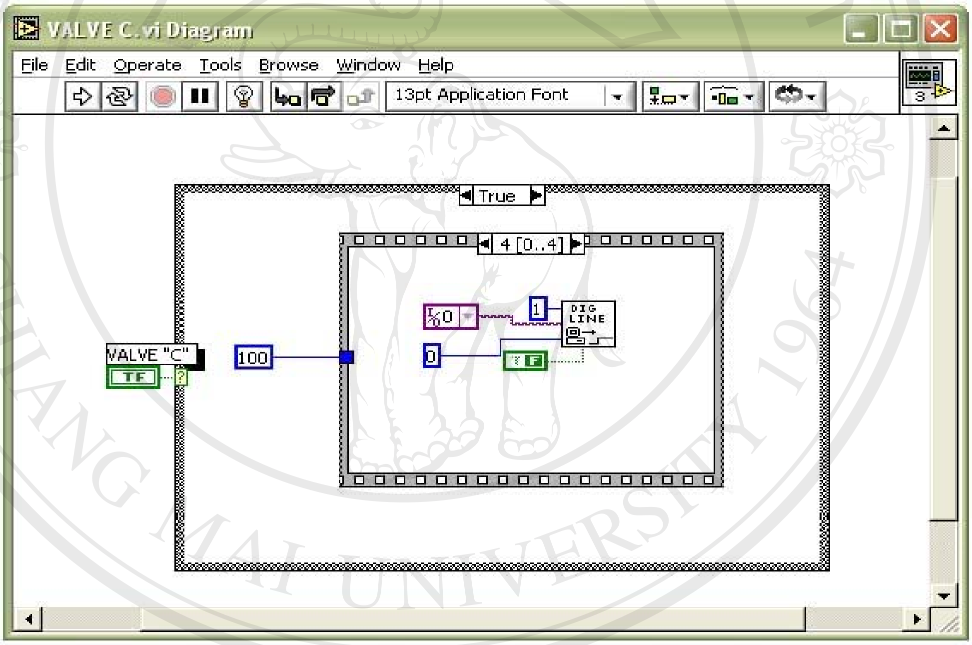
* flow rate 3.0 ml/min

2. LabView® computer program for controlling selection valve (V_1)

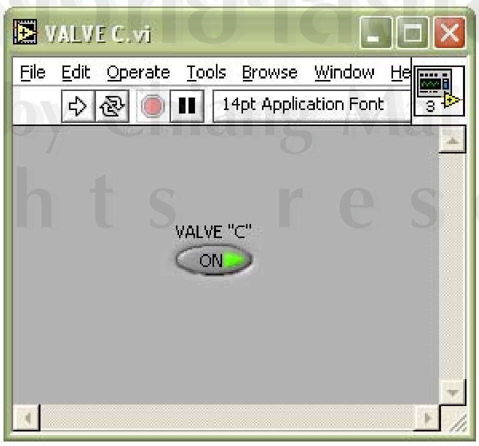


Note * refer Figure 2.5 page 24

2.1 Diagram of LabView® computer program for controlling selection valve (V_1)

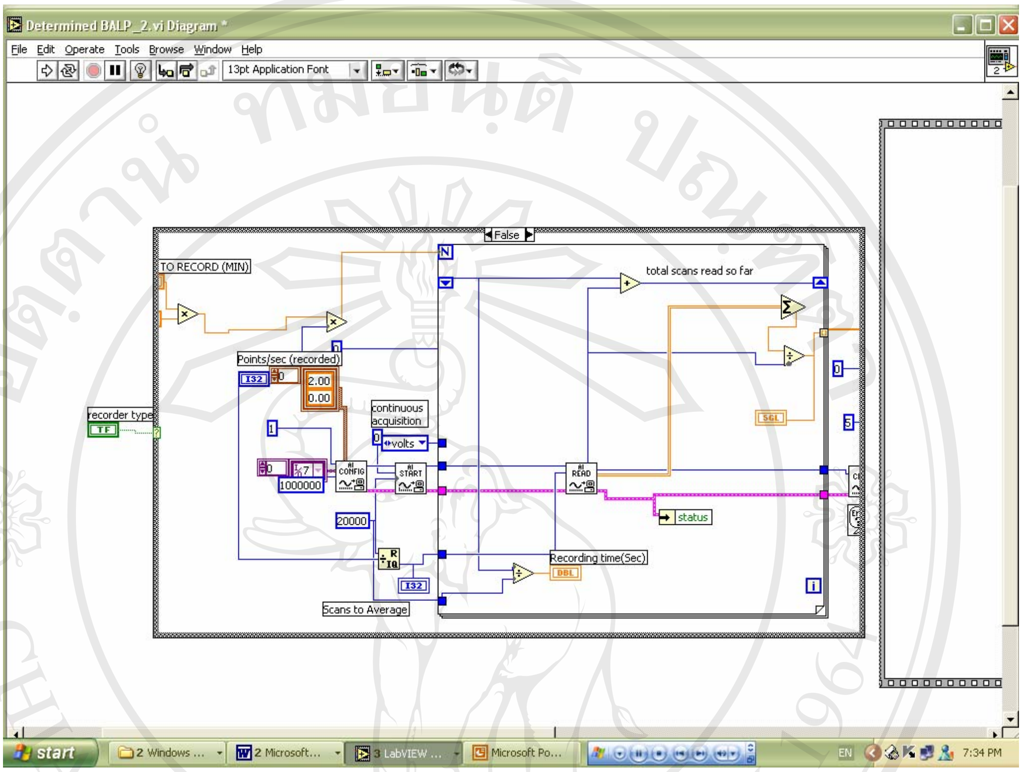


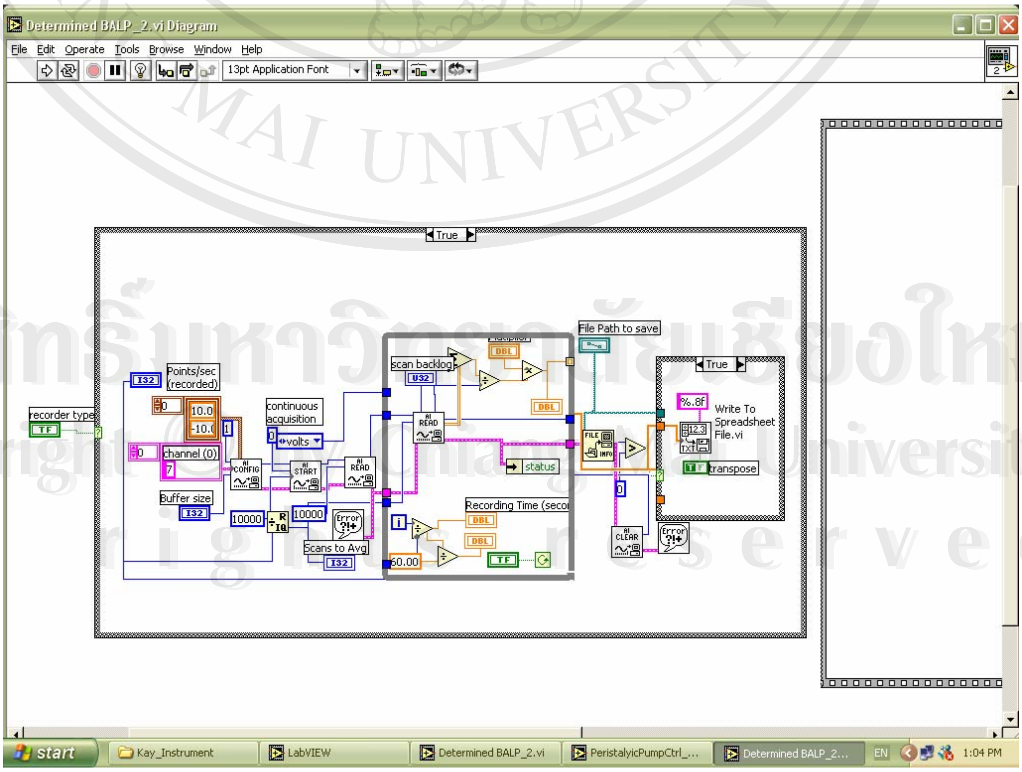
2.2 Display of LabView® computer program for controlling solenoid valve



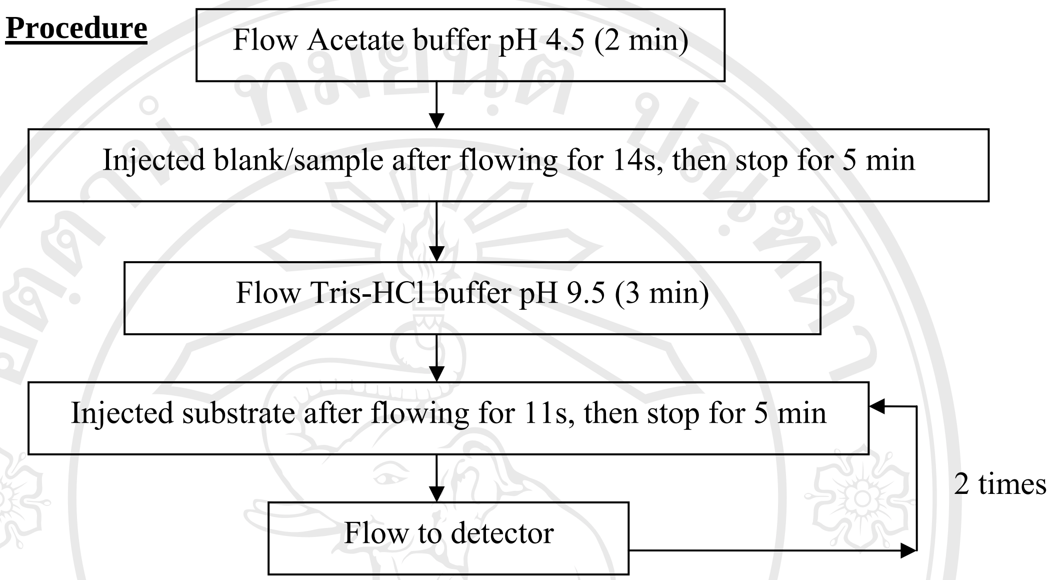
3. LabView® computer program for controlling the FI system

3.1 Diagram of LabView® for controlling the FI system

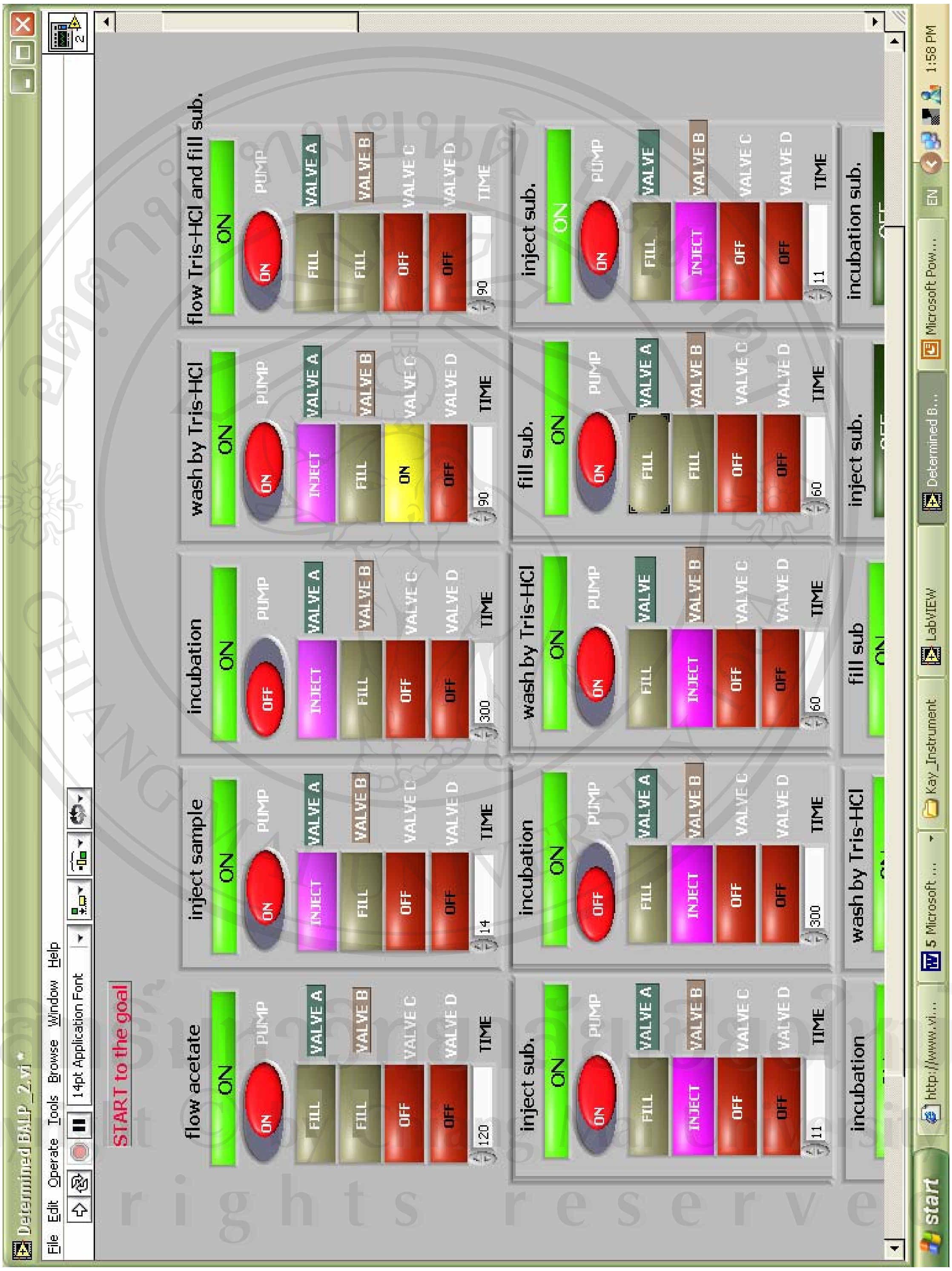


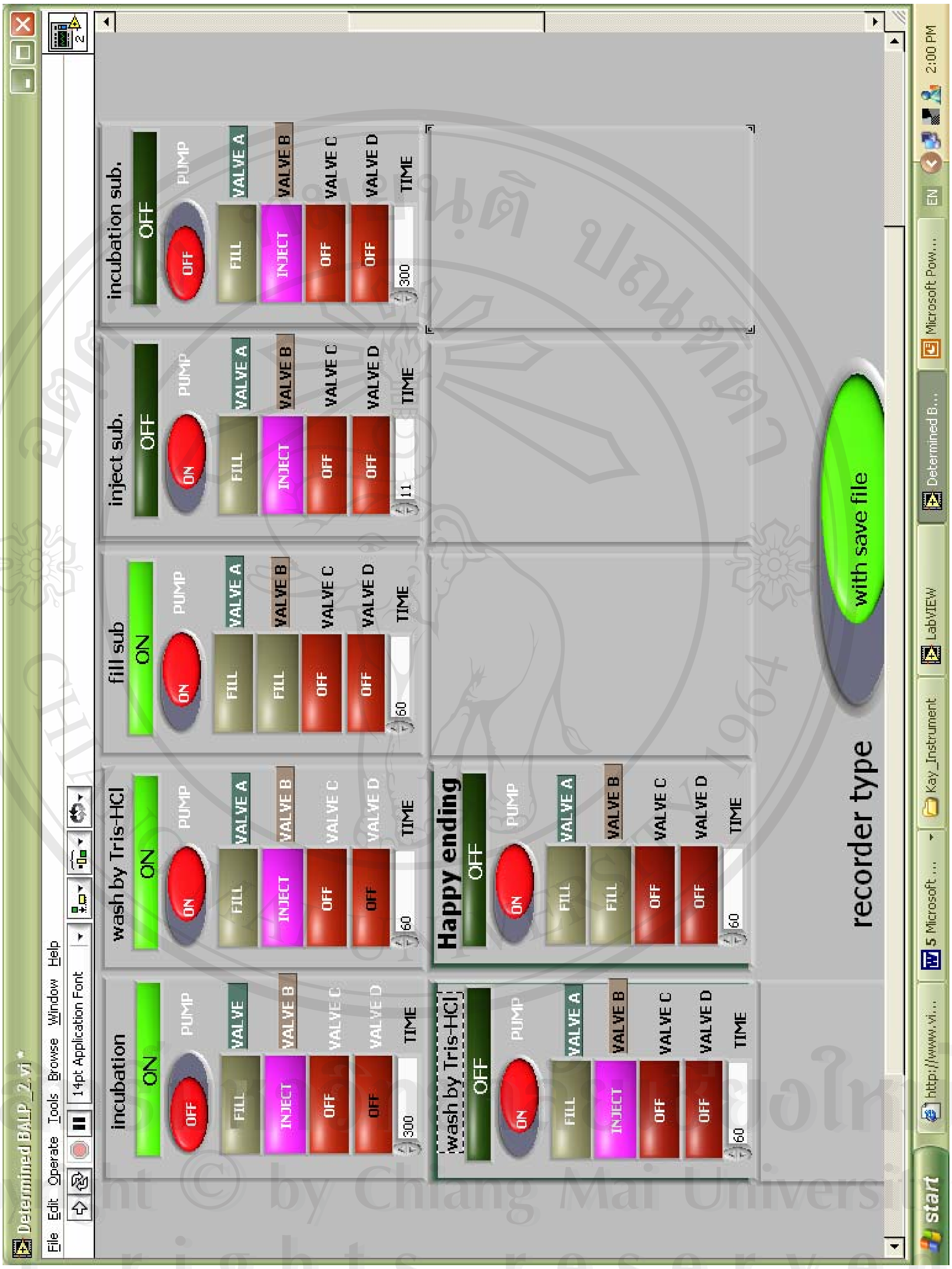


3.2 Display of LabView® computer program for controlling the FI-BI system

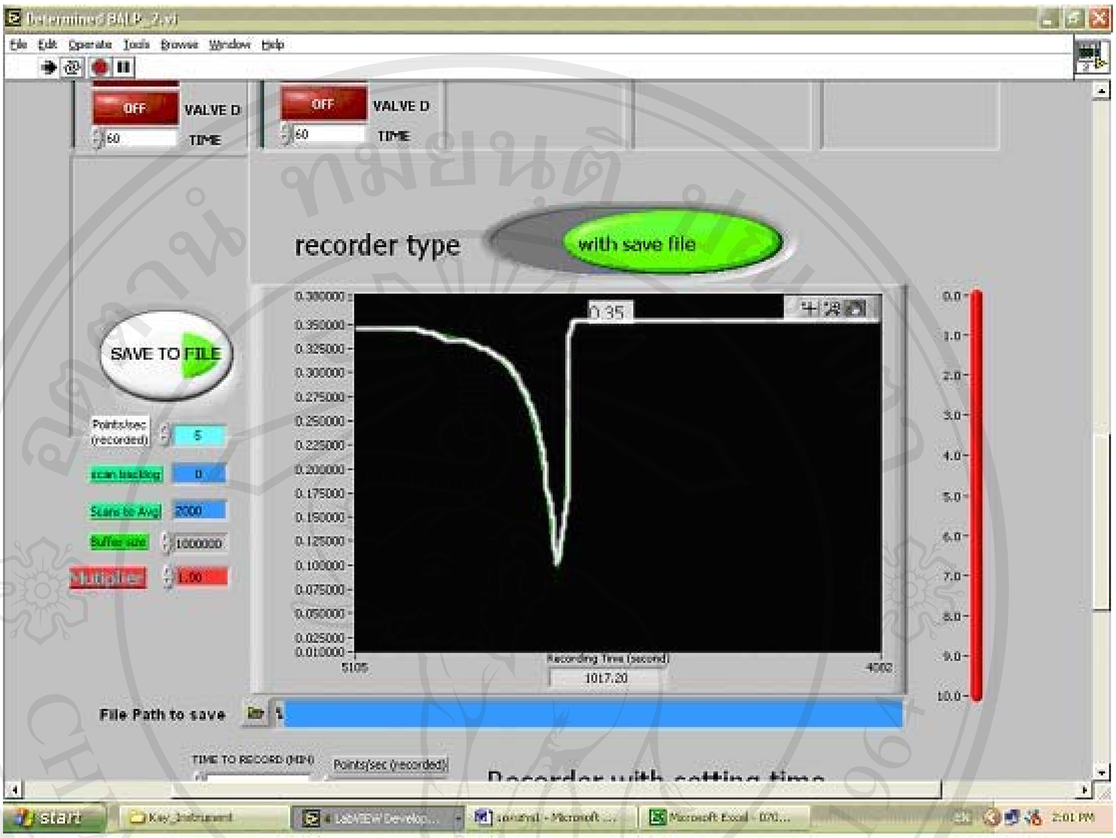


3.2.1 Display of operation control




Determined BALP_2.vi *
File Edit Operate Tools Browse Window Help
14pt Application Font
incubation
wash by Tris-HCl
fill sub
inject sub.
incubation sub.
wash by Tris-HCl
Happy ending
PUMP
VALVE A
VALVE B
VALVE C
VALVE D
TIME
FILL
INJECT
ON
OFF
recorder type
with save file
start

3.2.2 Display of recorder



APPEXDIX C

Diagram of Spectronic® 21 spectrophotometer

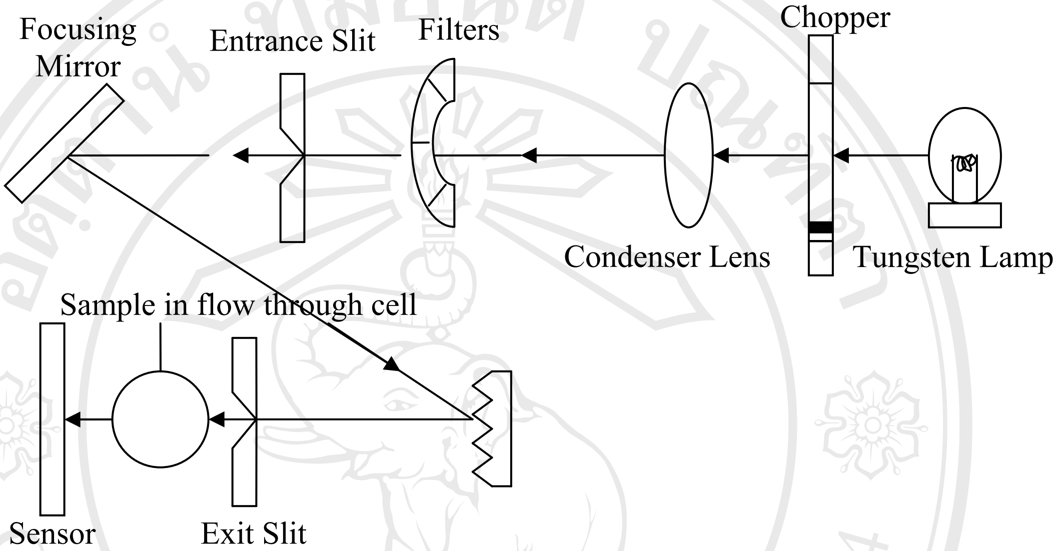
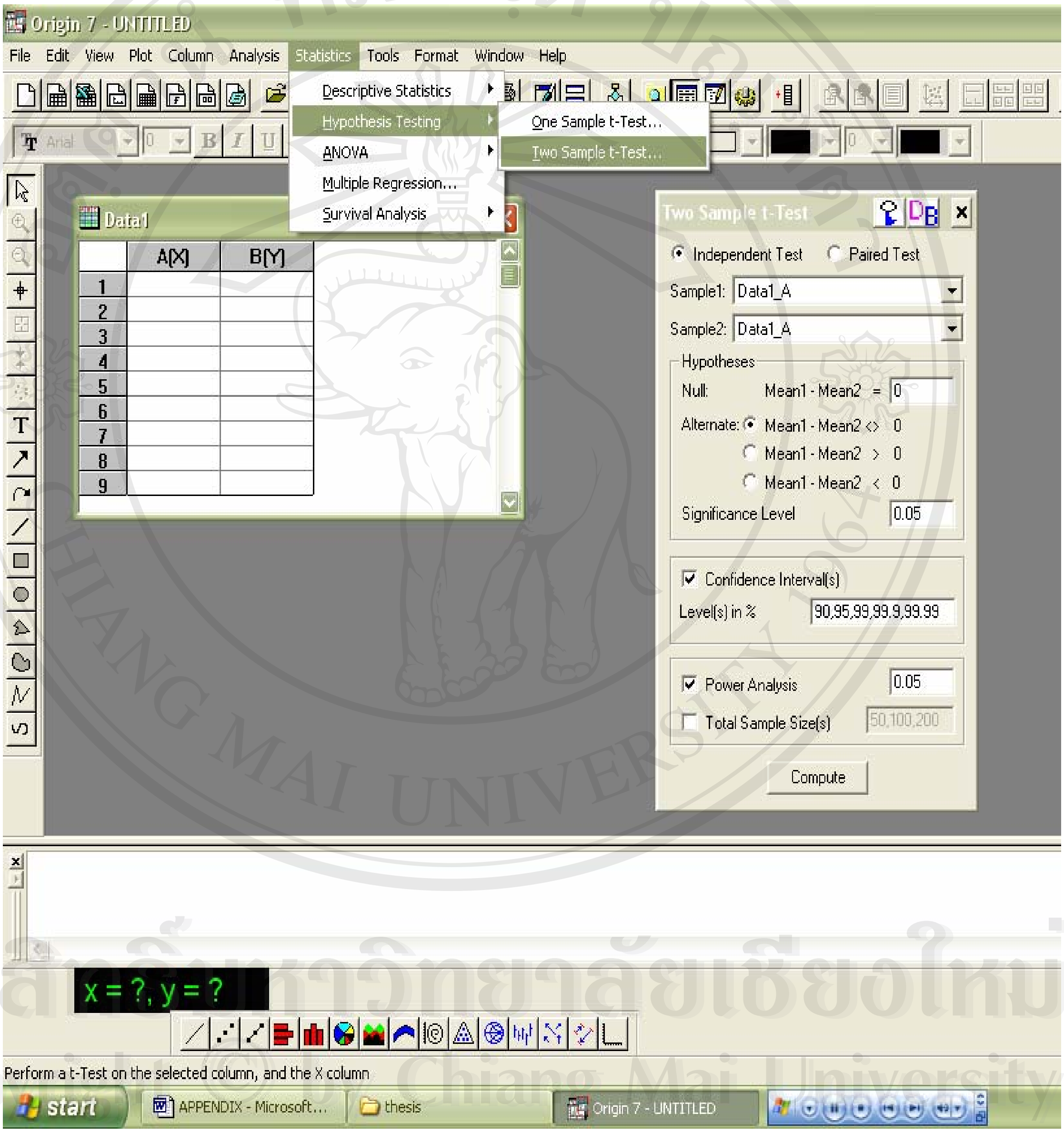


Figure C1 Optical diagram of the Spectronic® 21 spectrophotometer[32]

Light from the tungsten lamp is chopped by a chopper fan blade. The chopped light is focused on the entrance slit of the monochromator by a fused silica condenser lens. Four filters are positioned near the entrance slit which are used to eliminate higher order wavelengths and reduce stray light to a very low level. The filter are automatically programmed to intercept the light beam at the proper wavelengths. After passing through the slit, the light is incident on a focusing mirror and then converges onto the diffraction grating. The grating disperses the light and a spectrum is formed in the plane of the exit slit. As the grating is rotated, different wavelengths (or colors) become incident on the slit. The exit slit is rotated, different wavelengths (or colors) become incident on the slit. The exit slit isolates the selected narrow band of wavelengths. The exiting light then passes through the sample and is incident on the sensor. The sensor is a specially designed silicon chip which converts the light energy to an electrical signal for detection.

APPEXDIX D

Calculation of Two-Sample *t* Test by Origin computer program



1. Summary of Two-sample *t* test

Null Hypothesis: H_o : μ1-μ2 = hypothesized value

Test Statistic:

$$t = \frac{(mean_1 - mean_2) - (\mu_1 - \mu_2)}{[(S_1^2/n_1) + (S_2^2/n_2)]^{1/2}}$$

Where μ = population mean, is the average of all x (the variable for which we have sample data) values in the entire population.

S^2 = Variance, is the sum of squared deviations from the mean dicided by N-1.

n = the number of sample observation (sample size)

Alternative hypothesis	**Type of test**
H_a : μ1-μ2 > hypothesized value	Upper-tailed
H_a : μ1-μ2 < hypothesized value	Lower-tailed
H_a : μ1-μ2 ≠ hypothesized value	Two tailed

The appropriate degree of freedom (υ) for the two-sample *t* test is

$$\text{degree of freedom } (\upsilon) = \frac{[(S_1^2/n_1) + (S_2^2/n_2)]^2}{(S_1^2/n_1)^2/(n_1-1) + (S_2^2/n_2)/(n_2-1)}$$

Degree of freedom (υ) should be truncated (rounded down) to an integer.

H_o should be rejected if P-value ≤ α (significance level of the test)

H_o should not be rejected if P-value > α (significance level of the test)

In this research, two sample t-test was used for comparison of bone ALP activities in normal group (A) and abnormal group (B) from the FI-BI assay and commercial ELISA kit. Both groups are significantly different then null hypothesized (H_0) as zero was rejected (P-value ≤ α). The result was computed by Origin software as shown below.

Two sample t-test of bone ALP activities in normal group (A) and abnormal group (B) from the FI-BI assay

Two Sample Independent t-test

Summary Statistics

Sample	N	Mean	SD	SE
1. Data1_A	15	18.06667*	12.30254	3.1765
2. Data1_B	9	46.33333**	17.18284	5.72761

* refer to results from table in APPENDIX E (sample No. 1-15, bone ALP activity from FI-BI)

** refer to results from table in APPENDIX E (sample No. 16-24, bone ALP activity from FI-BI)

Difference of Means: -28.26667

Null Hypothesis: Mean1 - Mean2 = 0

Alternative Hypothesis: 0 < Mean1 - Mean2 > 0

t	DoF	P Value
-4.69746	22	1E-4

At the 0.0001 level, the difference of the population means is significantly different than the test difference (0).

Power Analysis

Alpha	Total Sample Size	Power	
1E-4	24	0.72929	(actual)

Two sample t-test of bone ALP activities in normal group (A) and abnormal group (B) from commercial ELISA kit

Two Sample Independent t-test

Summary Statistics

Sample	N	Mean	SD	SE
1. Data1_A	15	15.06667***	4.16562	1.07556
2. Data1_B	9	24.11111****	10.45759	3.48586

*** refer to results from table in APPENDIX E (sample No. 1-15, bone ALP activity from ELISA)

**** refer to results from table in APPENDIX E (sample No. 16-24, bone ALP activity from ELISA)

Difference of Means: -9.04444

Null Hypothesis: Mean1 - Mean2 = 0

Alternative Hypothesis: 0 < Mean1 - Mean2 > 0

t	DoF	P Value
-3.00932	22	0.00645

At the 0.01 level, the difference of the population means is significantly different than the test difference (0).

Power Analysis

Alpha	Total Sample Size	Power
0.01	24	0.57469 (actual)

2. Summary of the Paired *t* Test

Given two paired sets Xi and Yi of n measured values, the paired t-test determines whether they differ from each other in a significant level under the assumptions that the paired differences are independent and identically normally distributed.

To apply the test, let

$$\hat{X}_i = (X_i - \bar{X}) \quad (1)$$
$$\hat{Y}_i = (Y_i - \bar{Y}), \quad (2)$$

then define t by

$$t = (\bar{X} - \bar{Y}) \sqrt{\frac{n(n-1)}{\sum_{i=1}^{n} (\hat{X}_i - \hat{Y}_i)^2}}. \quad (3)$$

This statistic has n-1 degrees of freedom

H_o should be rejected if P-value $\leq \alpha$ (significance level of the test)

H_o should not be rejected if P-value $> \alpha$ (significance level of the test)

In this research, the paired t-test was used for method comparison between commercial ELISA kit (A) and FI-BI (B). Null hypothesized (H_0) was zero then both methods are not significantly different (P-value $> \alpha$). The result was computed by Origin software as shown below.

Two Sample Paired t-test

Summary Statistics

Sample	N	Mean	SD	SE
1. Data1_A	24	18.45833[+]	8.28293	1.69075
2. Data1_B	24	28.66667[++]	19.75429	4.03233

[+] refer to results from table in APPENDIX E (sample No. 1-24, bone ALP activity from ELISA)

[++] refer to results from table in APPENDIX E (sample No. 1-24, bone ALP activity from FI-BI)

Difference of Means: -10.20833

Null Hypothesis: Mean1 - Mean2 = 0

Alternative Hypothesis: 0 < Mean1 - Mean2 > 0

t	DoF	P Value
-3.40142	23	0.00245

At the 0.001 level, the difference of the population means is not significantly different than the test difference (0).

Power Analysis

Individual

Alpha	Sample Size	Power	
1E-3	24	0.35871	(actual)

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APPEXDIX E

The method comparison of the proposed FI-BI system with ELISA kit

sample No	BALP, U/L		Total ALP, U/L	ratio BALP / Total ALP	
	ELISA	FI-BI		ELISA	FI-BI
1	12±0.2	29±3	51	0.2	0.6
2	16±0.3	32±1	59	0.3	0.5
3	9±0.4	30±1	39	0.2	0.8
4	17±0.7	29±0	73	0.2	0.4
5	25±1.6	37±1	71	0.4	0.5
6	18±0.6	12±7	68	0.3	0.2
7	11±0.4	0±0	44	0.2	0.0
8	19±0.9	13±3	76	0.3	0.2
9	17±0.9	14±7	65	0.3	0.2
10	17±0.5	20±0	55	0.3	0.4
11	15±2.0	15±0	49	0.3	0.3
12	10±0.4	4±0	32	0.3	0.1
13	16±0.1	29±1	47	0.3	0.6
14	12±1.1	0±0	51	0.2	0.0
15	12±0.5	7±0	39	0.3	0.2
16	18±0.3	26±1	59	0.3	0.4
17	10±0.7	31±3	56	0.2	0.6
18	23±0.5	32±1	76	0.3	0.4
19	14±0.6	55±1	71	0.2	0.8
20	22±0.3	36±2	99	0.2	0.4
21	23±1.2	40±1	87	0.3	0.5
22	35±1.5	56±1	108	0.3	0.5
23	28±1.4	67±3	104	0.3	0.6
24	44±0.5	74±1			

Note: sample no 1-15: normal serum

sample no. 16-24: abnormal serum (osteoporosis)

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2003-2005 Full support from the Postgraduate Education and Research in Chemistry Program (PERCH)

PRESENTATIONS

1. D. Somprayoon, S. Kradtap Hartwell, P. Kongtawelert, S. Ong-chai, K. Grudpan, Flow Injection-Bead Injection System for Determination of Bone Alkaline Phosphatase (Poster Presentation), The International Symposium on Instrumentalized Analytical Chemistry and Computer Technology (InCom 2005), 29-31 March 2005, Dusseldof, Germany.
2. D. Somprayoon, S. Kradtap Hartwell, K. Grudpan, Flow Injection-Bead Injection System for Determination of Bone Alkaline Phosphatase (Poster Presentation), PERCH congress IV, 8-11 May 2005, Jomtein Palm Beach Resort, Pattaya, Chonburi, Thailand.
3. D. Somprayoon, S. Kradtap Hartwell, P. Kongtawelert, S. Ong-chai, S. Lapanantnoppakhun, K. Grudpan, Online Determination of Bone Alkaline Phosphatase Using Flow Injection-Bead Injection System (Oral and Poster Presentation), The 4th Annual Symposium on TRF Senior Research Scholar on "Development of Micro- and Nano-scale Analysis by Flow-based Technique I", 19 September 2005, Chiang Mai University, Chiang Mai, Thailand