APPENDIX A

Determination of fluoride in drug tablet with fluoride ion-selective electrode using a standard addition method [65]

1. Instrument and apparatus

1) Potentiostat: AUTOLAB® model PGSTAT 20 voltammetric analyzer (Eco Chemie, Utrecht, Netherlands), equipped with a VA Stand 663 (Metrohm, Herisau, Switzerland)
2) Silver/Silver Chloride reference electrode, Ag/AgCl (3M KCl), double-junction system with sleeve ceramic diaphragm
3) Fluoride-selective solid-state membrane electrode (Metrohm, Herisau, Switzerland)

2. Procedure

2.1 Determination of the slope of the fluoride calibration curve

Pipette 5.0 ml of standard solutions containing 2.0, 4.0, 6.0, 8.0 and 10.0 mg l^{-1} fluoride into a 10-50 ml vessel and add 5.0 ml of TISAB from a pipette. Stir the solution to ensure thorough mixing stop the stirrer, insert the fluoride ion selective electrode system and measure the potential. The electrode reaction rapidly becomes equilibrium, and the stable potential reading is obtained immediately.

Plot the observed potential against the varying concentrations of the standard solutions, use the log axis for the concentration, which should be in terms of fluoride ion concentration. A straight line plot (calibration curve) will be obtained according to equation A.1.

$$\text{Slope} = \frac{\Delta E}{\Delta(\log F)} \qquad (A.1)$$

2.2 Determination of fluoride concentration

Now take 5.0 ml (V_t) of sample solution, added 5.0 ml of TISAB and proceed to measure the potential, E_t, as above. Applying the usual Nernst equation, we can say

$$E_t = k_e + k \log y_t C_t \qquad \text{(A.2)}$$

where k_e is the electrode constant, k is theoretically 2.303 RT/nF but in practice it is the experimentally determined slope of the E *vs* log C plot for the given electrode, y_t and C_t are the activity coefficient and the concentration respectively of the ion to be determined in the test solution. A known volume V_2 (2×10^{-2} ml) of a standard solution (1000 mg l^{-1}; C_s) of fluoride is added to the test solution, and the new potential, E_2 is measured; For the new potential we can write:

$$E_2 = k_e + k \log y_t (V_t C_t + V_2 C_s)/(V_t + V_2) \qquad \text{(A.3)}$$

where V_t is the original volume of the test solution.

Provided that the first and second solutions are of similar ionic strength, the activity coefficient will be the same in each solution, and the difference between the two potential values can be expressed as

$$\Delta E = (E_1 - E_2) = k \log (V_t C_t + V_2 C_s)/C_t(V_t + V_2) \qquad \text{(A.4)}$$

This can be rearranged to give

$$C_t = C_s / [10^{\Delta E/k}(1 + V_t/V_2) - (V_t/V_2)] \qquad \text{(A.5)}$$

Hence provide the value of the slope constant (k) is known, the unknown concentration C_t can be calculated.

3. Results

3.1 Calibration graph of standard fluoride

The results of fluoride standard solutions containing 2.0, 4.0, 6.0, 8.0 and 10.0 mg l^{-1} are shown in Table A.1 and the calibration graph is illustrated in Figure A.1. It was found that the slop is –59.13.

Table A.1 Potentials of fluoride standard solutions (2.0-10.0 mg l^{-1})

F^- (mg l^{-1})	(log [F^-])	Potential (mV)			
		1	2	3	Mean
2.0	0.301	98.53	98.97	99.31	98.94
4.0	0.602	81.11	81.18	81.06	81.12
6.0	0.778	71.16	71.15	71.12	71.13
8.0	0.903	63.47	63.56	63.44	63.49
10.0	1.000	57.46	57.39	57.40	57.42

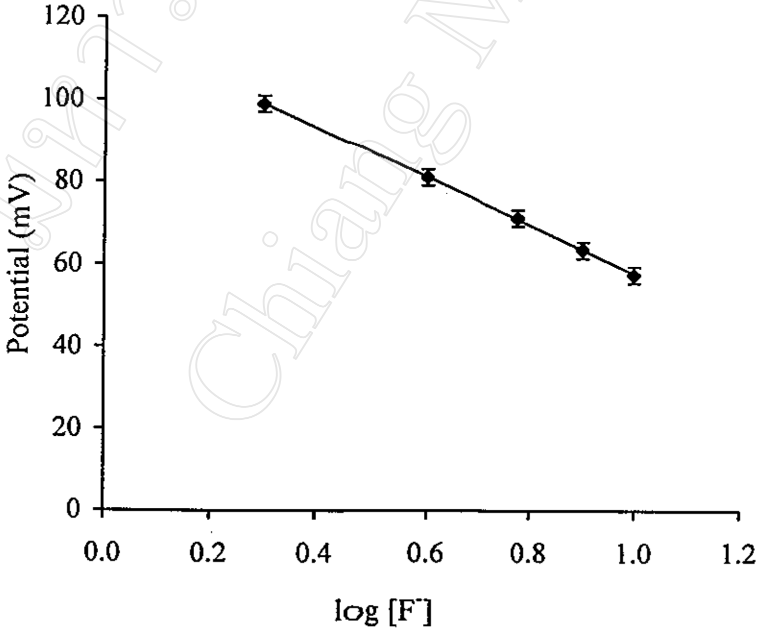

Figure A.1 Calibration graph of standard fluoride solutions (2.0-10.0 mg l^{-1})

3.2 Determination of fluoride in drug tablets

The result of fluoride determination in fluoride tablet samples by the fluoride ion selective electrode (ISE) with the standard addition method [65] are shown in Table A.2 and A.3

Table A.2 Triplicate measurement of potential for fluoride determination in fluoride tablet samples by the fluoride ion selective electrode (ISE) using a standard addition method [65]

Experiments	**Potential (mV)**					
	Sample No.1			**Sample No.2**		
	E_t	E_2	ΔE*	E_t	E_2	ΔE*
1	81.45	63.6	-17.85	82.06	65.03	-17.03
2	81.66	63.71	-17.95	81.98	64.08	-17.90
3	81.51	64	17.51	82.04	63.73	-18.31

*$\Delta E = E_2 - E_t$

Table A.3 Determination of fluoride in fluoride tablet samples by fluoride ion selective electrode (ISE) using a standard addition method [65]

Sample No.	**Fluoride (mg/tablet)**			**Mean (mg tablet^{-1})**	**SD**
	1	**2**	**3**		
1	0.25	0.24	0.25	0.25	0.006
2	0.97	0.98	0.97	0.97	0.006

4. Calculation

From the equation (A.5), the fluoride concentrations in sample solutions (C_t) are calculated.

$$C_t = C_s / [10^{\Delta E/k}(1+V_t/V_2)-(V_t/V_2)] \qquad (A.5)$$

Example of calculation.

4.1 Sample No.1

where k = slope of standard curve = -59.13

V_t = volume of sample solution = 5.0 ml

C_s = concentration of standard solution = 1,000 mg l^{-1}

V_2 = volume of a standard solution = $2.0\text{x}10^{-2}$ ml

ΔE = E_2-E_1 = 64.86-82.54 = -17.68 mV

Substitute the above value into equation A.5 and C_t can be calculated

$$C_t = 1000/\ [10^{-17.68/-59.13}(1+5/2\text{x}10^{-2})-(5/2\text{x}10^{-2}]$$
$$= 4.005 \text{ mg } l^{-1}$$

The fluoride contents per tablet (in mg tablet^{-1}) can be calculated as follow:

- concentration of sample solution = 4.005 mg l^{-1}
- concentration of stock sample solution = 4.005x100 = 400.5 mg l^{-1}
- mg of fluoride in 50.0 ml of stock sample solution
  = 400.5 x 50 /1000 = 20 mg
- mg of fluoride in 1 tablet = 20 mg x 0.0403 /3.2530
  = 0.2477 mg *Ans*

(average weight tablet^{-1} = 0.0403 g and fluoride powder = 3.2530 g)

4.2 Sample No.2

k = slope of standard curve = -59.13

V_t = volume of sample solution = 5.0 ml

C_s = concentration of standard solution = 1,000 mg l^{-1}

V_2 = volume of a standard solution = $2.0\text{x}10^{-2}$ ml

ΔE = E_2-E_1 = 63.95-82.02 = -18.07 mV

Substitute the above value into equation A.5 and C_t can be calculated

$$C_t = 1000/\,[10^{-18.07/-59.13}(1+5/2\text{x}10^{-2})-(5/2\text{x}10^{-2}]$$
$$= 3.886 \text{ mg } l^{-1}$$

The fluoride contents per tablet (in mg tablet^{-1}) can be calculated as follow:

- concentration of sample solution = 3.886 mg l^{-1}
- concentration of stock sample solution = 3.886x100 = 388.6 mg l^{-1}
- mg of fluoride in 50.0 ml of stock sample solution
  = 388.6 x 50 /1000 = 19.43 mg
- mg of fluoride in 1 tablet = 19.98 mg x 0.0408 /0.8150
  = 0.9726 mg *Ans*

(average weigh tablet^{-1} = 0.0408 g and fluoride powder = 0.8150 g)

APPENDIX B

Glycolic acid determination in urine sample by HPLC [87]

1. Instrument, apparatus and conditions

1. Column ; Series column configulation consisted of a Nova-Pack C18 (4mm), 3.9 x150 mm, (Waters) and a Spherisorb ODS (5 mm), 4.6 x 150 mm (Phenomenex)
2. Pump ; Binary LC Pump 250, Perkin-Elmer
3. Detector ; LC-95 UV/Visible spectrometer, λ=210 nm , 0.1 AUFS , Perkin-Elmer.
4. Cellulose acetate membrane (0.4µm)
5. Sample volume ; 20 µl (Rheodyne injection valve)
6. Mobile phase ; Sulphuric acid, pH 3.0, 0.3 ml/min

2. Procedure

2.1 Calibration graph of glycolic acid standard solution

A 10.0 ml of glycolic acid standard solutions was mixed with 300 mg of activated charcoal and the charcoal was removed by centrifugation. The supernatant was passed through a cellulose acetate membrane (0.4 µm) before injected to HPLC system.

2.2 Glycolic acid determination in Urine sample

A 10 ml of 10-fold diluted sample was mixed with 300 mg of activated charcoal and the charcoal was removed by centrifugation. The supernatant was passed through a cellulose acetate membrane (0.4 µm) before injected into the HPLC system.

3. Results

3.1 Calibration graph of glycolic acid standard solution for comparing with FI system (section 3.2.4)

The results of glycolic standard solution (0.5, 1.0, 2.0 and 3.0 mM) is shown in Figure A.2 and Table A.4. The calibration graph was illustrated in Figure A.3.

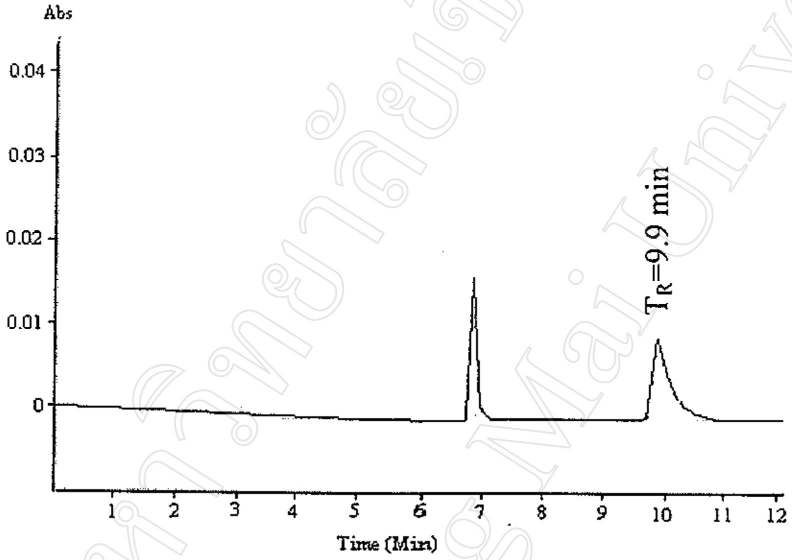

Figure A.2 Chromatogram of 1.0 mM standard glycolic acid

Table A.4 High performance liquid chromatographic determination of glycolic acid in standard solution containing 0.5, 1.0, 2.0 and 3.0 mM glycolic acid for comparing with those obtained by FIA system (section 3.2.4)

Glycolic acid (mM)	**Peak height (Abs)**			**Mean (Abs)**	**SD**
	1	**2**	**3**		
0.0	0.0000	0.0000	0.0000	0.0000	0.0000
0.5	0.0055	0.0052	0.0054	0.0054	0.0002
1.0	0.0120	0.0119	0.0120	0.0120	0.0001
2.0	0.0224	0.0223	0.0222	0.0223	0.0001
3.0	0.0335	0.0335	0.0333	0.0334	0.0001

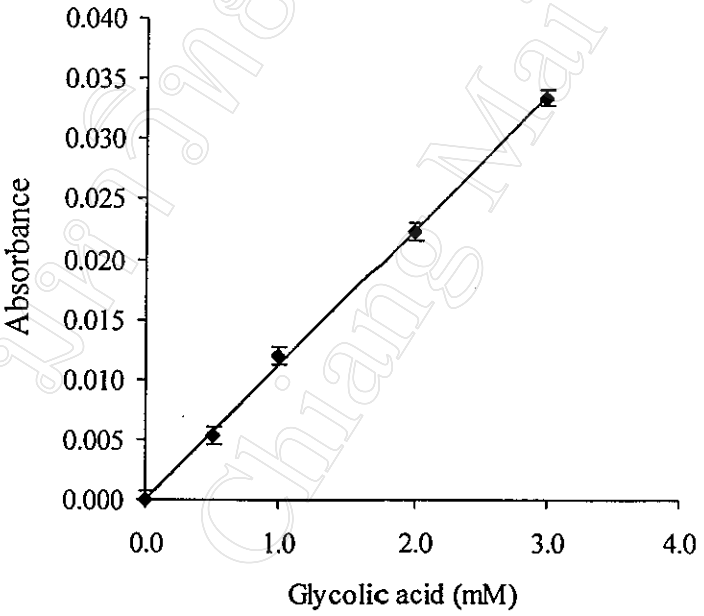

Figure A.3 Calibration graph for glycolic acid (0.5-3.0 mM)

The results of glycolic acid determination in 10 fold-dilution of pretreated urine samples by HPLC are shown in Table A.5 and the example of chromatogram of the urine sample is shown in Figure A.4.

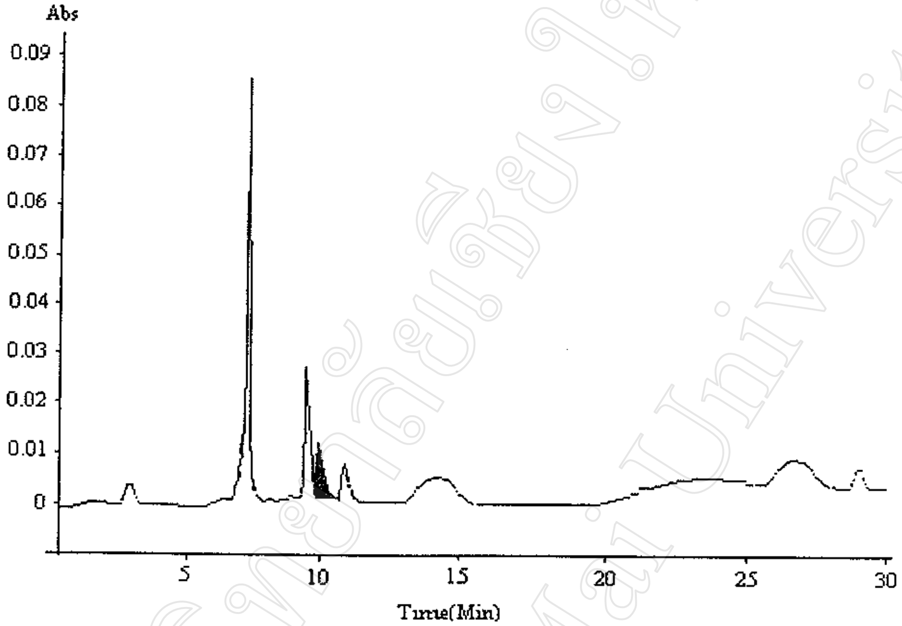

Figure A.4 Chromatogram of in 10 fold-dilution of pretreated urine samples

Table A.5 Results of glycolic acid determination in in 10 fold-dilution of pretreated urine samples by HPLC for comparing with those obtained by FIA system (section 3.2.4)

Sample No.	**Glycolic acid (mM)**			**Mean (mM)**	**SD**
	1	**2**	**3**		
1	0.48	0.50	0.49	0.49	0.0089
2	0.71	0.71	0.71	0.71	0.0052
3	0.7	0.71	0.72	0.71	0.0136

3.2 Calibration graph for glycolic acid determination in urine samples for comparing with those obtained by BI system (section 3.3.4)

Peak heights of glycolic standard solution containing 0.1, 0.5, 1.0, 1.5 and 2.0 mM glycolic acid are shown in Table A.6 and the calibration graph is illustrated in Figure A.5.

Table A.6 Peak heights of 0.1, 0.5, 1.0, 1.5 and 2.0 mM glycolic acid for construction of calibration graph for glycolic acid determination comparing with those obtained by BIA system (section 3.3.4)

Glycolic acid (mM)	**Peak height (Abs)**			**Mean(Abs)**	**SD**
	1	**2**	**3**		
0.1	0.0010	0.0010	0.0010	0.0010	0.0000
0.5	0.0050	0.0050	0.0050	0.0050	0.0000
1.0	0.0100	0.0100	0.0100	0.0100	0.0000
1.5	0.0157	0.0156	0.0151	0.0155	0.0003
2.0	0.0216	0.0215	0.0213	0.0215	0.0002

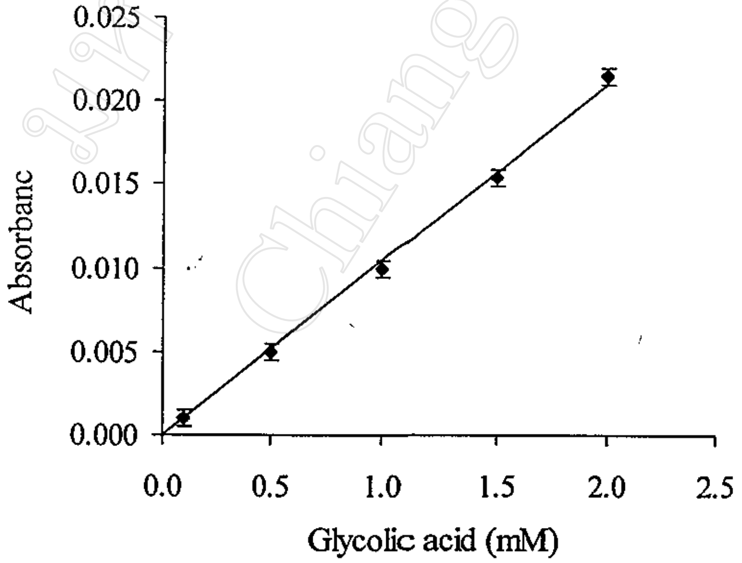

Figure A.5 Calibration graph for glycolic acid (0.1-2.0 mM)

The results of glycolic acid determination in in 10 fold-dilution of pretreated urine samples by HPLC are shown in Table A.7.

Table A.7 Result of glycolic acid determination in 10 fold-dilution of pretreated urine samples by HPLC for comparing with those obtained by BI system (section 3.3.4)

Sample No.	**Glycolic acid (mM)**			**Mean (mM)**	**SD**
	1	**2**	**3**		
1	0.19	0.18	0.19	0.19	0.0055
2	0.35	0.33	0.33	0.34	0.0110
3	0.37	0.34	0.32	0.35	0.0240
4	0.43	0.42	0.42	0.42	0.0055
5	0.48	0.47	0.47	0.47	0.0055

VITA

Name: Mr. Prasit Purachat

Date of birth: July 27, 1960

Acadamic status:

- B.Ed. (General Science), Srinakharinwirot University, 1985.
- M.S. (Teaching chemistry), Chiang Mai University, 1988.
- Ph.D. student in analytical chemistry, Chiang Mai University, 1997-2002.

Practical experiences:

- Teacher, Sena"Senaprasit" School, Ayuthaya, 1981-1994.
- Lecturer, Department of Chemical, Faculty of Science and Technology, Rajabhat Institure Thepsatri, Lopburi, 1994-present.

Awards/Scholarships:

- The Postgraduate Education and Research Program in Chemistry (PERCH).

List of publications:

- S. Liawruangrath, W. Oungpipat, S. Watanesk, B. Liawruangrath, C. Duangduen, P. Purachat, "Asparagus based amperometric sensor for fluoride determination", *Anal. Chim. Acta*, **448** (2001) 37-46.

List of conferences

1. B. Purachat and P. Purachat, "*Using of simplex method for the selection of parameter for The Spectrophotometric Determination Of Sulphate in Water*", 24th Congress on Science and Technology of Thailand, Bangkok, 1998.
2. P. Purachat, S. Liawrungrath, W. Oungpipat, S. Watanesk, B. Liawrungrath, C. Duangduen, *"Plant Tissue-Based Electrode or The Determination of Fluoride*", 26th Congress on Science and Technology of Thailand, Bangkok, 2000.
3. P. Purachat, S. Liawruangrath, W. Oungpipat, S. Watanesk, B. Liawruangrath, C. Dongduen, "*Sunflower Leaves Electrode for The Determination of Glycolic Acid*", 27th Congress on Science and Technology of Thailand, Songkhla, 2001.