CHAPTER III

EXPERIMENTS

This investigation dealed with the factors affecting the accuracy and precision of potentiometric titration of weak acid mixtures by multiple linear regression analysis. There were many factors involving in this research such as : the difference between pKa of each weak acids, the initial concentration ratios of both weak acids, the standard errors of measurement of both pH observed and volume of base used, the changes of ionic strength of solution during the course of titration, the titration data range being interpreted, the experimental condition and the applicability and limitation of the multiple linear regression analysis computer program (SPSS/PC$^+$). These factors affected both the accuracy and precision of the resulting values of equivalent volumes.

1. The applicability and limitation of SPSS/PC$^+$ program.

The computer curve fitting program, SPSS/PC$^+$, was used for interpreting the data. To test the applicability of the program, it was necessary to prove theoretically that this program could be used to solve the modified equation for equivalent volume determination of weak acidic

mixture by simulation the data for comparative purpose with experimental data.

The set of data were synthesized by solving the polynomial equation at any given values of the following parameters with assumption that the inoic strength of the solution was constant throughout the course of titration, the parameters were as followed:

1. Dissociation constant of acid A and acid B (Ka_A, Ka_B)
2. Initial concentration of both acid A and acid B
3. Ionization product constant of water (Kw)
4. Concentration of titrant (normality of sodium hydroxide)
5. Initial volume of solution being titrated (Vo)

The set of data was the concentration of hydronium ion as the function of the volume of titrants. The principle of polynomial equation was based on mass balance, charge balance and equilibrium equation and could be derived, as followed.

The initial steps of derivation were in the same pattern as for the derivation of Equation 42.

$$[H^+] + VN - [OH^-] = \frac{Ka_A Ve_A N}{(Vo+V)(Ka_A+H^+)} + \frac{Ka_B Ve_B N}{(Vo+V)(Ka_B+H^+)} \quad \text{Eq.193}$$

Multiplying Eq.93 with $[H^+](V+Vo)$ gave

$$[H^+]^2(V+Vo) + VN[H^+] - Kw(V+Vo) = \frac{Ka_A Ve_A N[H+]}{(Ka_A+[H+])}$$

$$+ \frac{Ka_B Ve_B N[H+]}{(Ka_B+[H+])} \qquad \text{Eq.194}$$

Multiply Eq.194 with $(Ka_A + [H^+])(Ka_B + [H^+])$ gave

$$[H^+]^2(V+Vo)(Ka_A + [H^+])(Ka_B + [H^+])$$
$$+ VN[H^+](Ka_A + [H^+])(Ka_B + [H^+])$$
$$- Kw(V+Vo)(Ka_A + [H^+])(Ka_B + [H^+]) =$$
$$Ka_A Ve_A N[H+](Ka_A + [H^+])(Ka_B + [H^+])$$
$$+ Ka_B Ve_B N[H+](Ka_A + [H^+])(Ka_B + [H^+]) \qquad \text{Eq.195}$$

Eq.195 could be rearranged to

$$[H^+]^4$$
$$+ \{Ka_A + Ka_B + VN/(Vo+V)\}[H^+]^3$$
$$+ \left\{Ka_A Ka_B + \frac{(Ka_A + Ka_B)VN}{(Vo+V)} - Kw - \frac{Ka_A Ve_A N}{(Vo+V)} - \frac{Ka_B Ve_B N}{(Vo+V)}\right\}[H^+]^2$$
$$+ \left\{\frac{Ka_A Ka_B VN}{(Vo+V)} - (Ka_A + Ka_B)Kw - \frac{Ka_A Ka_B Ve_A N}{(Vo+V)} - \frac{Ka_A Ka_B Ve_B N}{(Vo+V)}\right\}[H^+]$$
$$- Ka_A Ka_B Kw = 0 \qquad \text{Eq.196}$$

Eq.196 could be written in polynomial form

$$A4[H^+]^4 + A3[H^+]^3 + A2[H^+]^2 + A1[H^+] + A0 = 0 \quad \text{Eq.197}$$

where

A4 = 1

A3 = {KaA + KaB + VN/(Vo+V)}

$$A2 = \{Ka_A Ka_B + \frac{(Ka_A + Ka_B)VN}{(Vo+V)} - Kw - \frac{Ka_A Ve_A N}{(Vo+V)} - \frac{Ka_B Ve_B N}{(Vo+V)}\}$$

$$A1 = \{\frac{Ka_A Ka_B VN}{(Vo+V)} - (Ka_A + Ka_B)Kw - \frac{Ka_A Ka_B Ve_A N}{(Vo+V)} - \frac{Ka_A Ka_B Ve_B N}{(Vo+V)}\}$$

A0 = Ka_A Ka_B Kw

When each of weak acid or both of weak acids were in the ionized form, the polynomial equation could be derived in the same manner when both weak acids were in the neutral form and the polynomial equation was identical to Eq.197.

The variable in polynomial equation (Eq.197) was hydronium ion concentration. The parameters in the coefficient (A0-A4) of polynomial equation (Ka_A, Ka_B, V_{EA}, V_{EB}, N, V_o and Kw) were fixed except the volume of titrants. Thus, by solving the polynomial equation giving the set of data, which was the hydronium ion concentration as a function of the volume of titrant. Newtons' approximation method (Butler, 1964 and Greenspan, 1988) and computer program, Macro Lotus were used in order to solve this polynomial equation. This Newton's approximation method was also described in the textbooks of Maron

(1987); Maron and Lopez (1991); Mendenhall, Scheaffer and Wackerly (1981) and Pizer (1983). Each set of data, hydronium ion concentration as a function of the volume of titrant, was obtained from changing the pKa of each weak acid and the initial concentration ratios of both acid in the polynomial equation, which could give the difference of ΔpKa and initial concentration ratios (X) as shown in Table 2. The sets of data (hydronium ion concentration as a function of the volume of titrant, obtained from simulation) were the same character as obtained from the titration in the real experiment. The set of data, obtained from simulation, was applied to the modified equation (Eq.42) for calculation of Ve_A and Ve_B values with the use of multiple linear regression analysis and SPSS/PC$^+$ program.

2. The accuracy of potentiometric titration of two weak acid mixtures by multiple linear regression analysis.

2.1 Modification of equations used to find the equivalent volume of weak acid mixtures.

By introduction of the thermodynamic dissociation constant to the equations based on the basic principle of mass balance, charge balance and equilibrium equation, the modified equations were improved. The derivation of these equations was described previously.

These equations (Eq.106,123,134,148,158,159) were used to determine the equivalent volumes of each single acid from the two weak acid mixture titration.

2.2 Equipment :

1) pH meter (ORION 940 pH/ISE meter)
2) Combined glass electrode (ORION 9172 BN)
3) Automatic titrator and exchange unit (ORION 960 auto chemistry system.).

2.3 Materials

The weak acids chosen in the experiment were based on these criteria :

1) Weak acid of which pKa was between 4 and 9.
2) Both form of the weak acids were selected :
   Neutral weak acid and Ionized weak acid.
3) Soluble in 0.1 M potassium chloride solution and not precipitate during the course of titration.
4) Stable throughout the titration experiment.
5) The interaction between those weak acids selected had not to occur in the titration solution.

Those weak acids selected in this investigation were as followed:

1. Neutral weak acids

1.1 <u>Benzoic acid</u>, AR (M&B, Lot # 57518)

Empirical formula	:	$C_7H_6O_2$
Molecular weight	:	122.12
pKa (28°C)	:	4.060
Description	:	monoclinic tablets, plates, leaflets
Solubility (Budavari, ed., 1989)		
	:	one gram dissolves in 2.3 ml ether, 3 ml acetone, 10 ml benzene, 30 ml carbon disulfide, 23 ml oil of turpentine, 250 ml water (25°C) and 230 ml water (30°C).

1.2 <u>Pivalic acid</u>, (Sigma, Lot # 43H3747)
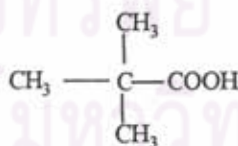
Empirical formula	:	$C_5H_{10}O_2$
Molecular weight	:	102.13
Description	:	colorless, needle crystal, stable in air
pKa (28°C)	:	5.010
Solubility (Budavari, ed., 1989)		

: one gram dissolves in 40 ml water, freely soluble in alcohol, ether.

1.3 p-Nitrophenol, AR (Fluka Chemica, Lot # 73560)
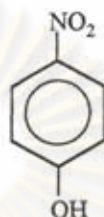
Empirical formula	:	$C_6H_6NO_2$
Molecular weight	:	139.11
Description	:	colorless to slightly yellow, odorless crystals, sweetish, then burning taste.
pKa (28°C)	:	7.011

Solubility (Budavari, ed., 1989)

: moderately soluble in cold water, freely soluble in alcohol, chloroform, ether, also soluble in solution of fixed alkali hydroxide and carbonates.

1.4 Boric acid, AR (E. Merck, Lot # 5690020)

Empirical formula	:	H_3BO_3
Molecular weight	:	61.84

Description   : colorless, odorless, transparent crystals, or white granules or powder
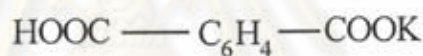
pKa (28°C)   : 9.096

Solubility (Budavari, ed., 1989)

: one gram dissolves in 18 ml cold water, 4 ml boiled water, 6 ml boiled alcohol and 4 ml glycerol.

2. Ionized weak acids

2.1 Potassium biphthalate, AR (Fluka Chemica, Lot # 266270)

$$HOOC \text{——} C_6H_4 \text{—} COOK$$

Empirical formula   : $C_8H_5KO_4$

Molecular weight   : 204.23
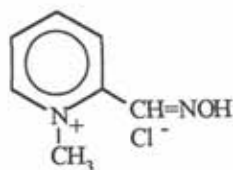
Description   : orthorhombic crystal, stable in air

pKa (28°C)   : 5.005

Solubility (Budavari, ed., 1989)

: one gram dissolves in about 12 part cold water and 3 part boiling water.

2.2 Pralidoxime chloride, (Sigma, Lot # 60H0445)

Empirical formula   : $C_7H_9N_2O\ Cl$

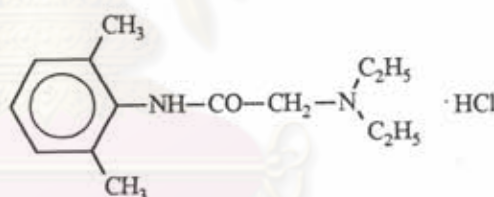Molecular weight   :   172.63

Description (Florey, ed., 1988)

                           :   crystalline, odorless, stable in air at tempetature below 100°C, white to pale yellow.

pKa (28°C)   :   7.939

solubility (Budavari, ed., 1989)

                           :   one gram dissolves in 2 ml water, 12 ml methanol, 100 ml ethanol and 1000 ml isopropanol.

2.3 Lidocaine hydrochloride, (Sigma, Lot # 62H0959)

Empirical formula   : $C_{14}H_{22}N_2O\ HCl$

Molecular weight   :   270.82

Description (Florey, ed., 1985)

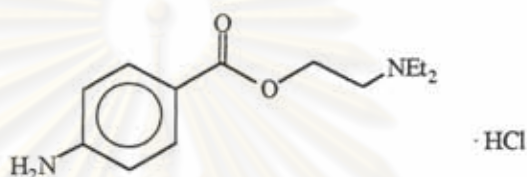   :   both the based and hydrochloride are white odorless substance.

pKa (28°C)   :   7.954

Solubility (Budavari, ed., 1989)

                           :   HCl salt : 0.68 gram in 1 ml water.

free base : 0.004 gram in 1 ml water,

0.76 gram in 1 ml 95% ethanol,

0.79 gram in 1 ml chloroform,

0.12 gram in 1 ml n-hexane.

2.4 Procaine hydrochloride, AR

Empirical formula	:	$C_{13}H_{20}N_2O_2$ HCl
Molecular weight	:	272.8
Description	:	colorless crystals or a white crystalline powder, odorless.
pKa (28°C)	:	9.070

Solubility (Budavari, ed., 1989)

: HCl salt : one gram dissolves in 1 ml water, 30 ml 96% ethanol , slightly soluble in chloroform, practically insoluble in ether.

free base : one gram dissolves in 200 ml water, soluble in alcohol, ether, benzene and chloroform.

2.4 Solvent and Other Reagents :

1) Sodium hydroxide, AR (E. Merck, Lot # 327 K19742898)

2) Potassium chloride, AR (E. Merck, Lot # 208 TA253636)

3) Distilled water (freshly prepared)

4) Electrode filling and storage solution, 4 M KCl saturated with AgCl, (ORION, Lot # 900011)

5) Standard buffer solution pH 4 (ORION, Lot # 910104)

6) Standard buffer solution pH 7 (ORION, Lot # 910107)

7) Standard buffer solution pH 10 (ORION, Lot # 910110)

2.5 Preparation of 0.1N sodium hydroxide standard solution

Sodium hydroxide was dissolved in an equal weight of water and allowed to stand overnight in close container for preventing absorption of carbon dioxide. Clear supernatant liquid was decanted and diluted from 5.5 ml with carbon dioxide-free water to produce 1000 ml (Recommendation of the Medicine Commission, 1988).

2.6 Standardization of sodium hydroxide solution

Sodium hydroxide solution was standardized with 50 mg potassium biphthalate (dried at 120°C, 2 hours) in 50 ml of carbon

dioxide-free water. One ml of 1.000 N sodium hydroxide was equivalent to 204.22 mg of potassium biphthalate. (Recommendation of the Medicine Commission, 1988).

2.7 Preparation of weak acidic compounds in 0.1 M KCl

Potassium chloride was dissolved and diluted with distilled deionized water to give a final concentration about 0.1 M. Each weak acidic compound was accurately weighed in suitable quantities (according to its molecular weight) to produce two folds of its required concentration. Then, they were dissolved in the solution of 0.1 M potassium chloride in order to control the ionic strength of the solution. The weak acidic compound solution 25.0 ml and 0.1 M KCl 25.0 ml were transferred to a 150 ml beaker to obtain its required concentration.

2.8 Preparation of two-mixed weak acidic compounds in 0.1 M KCl

Each 25.0 ml of two weak acidic compound solutions whose concentration was about two folds of the required concentration was transferred to a 150 ml beaker to obtain its required concentration.

2.9 Titration of single weak acid solution and two-mixed weak acids solution with 0.1 N sodium hydroxide.

For prevention of carbon dioxide absorption during titration, the experiments were performed in closed vessel with the way to pass nitrogen gas over the solution throughout the experiment. Electrode was recalibrated with buffer each time before titration and submerged into titrated solution for 5 minutes to assure equilibration. It was necessary to beware not to rinse the side of the beaker with distilled water since the exact volume of solution had to carefully controlled. Raw data of experiments were obtained from five determinations of each sample solution.

2.10 The steps of analyzing data for determining the equivalent volumes

1. From the raw data obtained from the titration of each single weak acid solution, the equivalent volume could be determined by the extrapolation of linear plot of modified Gran methods according to Eq. 67, Eq.79 and Eq.89.

The slope of this plot was the thermodynamic dissociation constant (Ka^o) at temperature 28^oC. The equivalent volumes of each weak acid could determined from the intercept. The data range interpreted was the range which gave maximum r^2 as demonstrated in Figure 2 and 3.

where V_{iA} = the initial volume of titrant of G plot linearity range of single weak acid A (stronger acid).

V_{fA} = the final volume of titrant of G plot linearity

range of single weak acid A (stronger acid).

V_{iB} = the initial volume of titrant of G plot linearity

range of single weak acid B (weaker acid).

V_{fB} = the final volume of titrant of G plot linearity

range of single weak acid B (weaker acid).

pH_{maxA} = maximum pH of G plot linearity range of acid A.

pH_{maxB} = maximum pH of G plot linearity range of acid B.

2. From the raw data obtained from the titration of two-mixed weak acid solution, the equivalent volumes could be determined by using the multiple linear equations which were derived in terms of $y = a_1x_1 + a_2x_2$ according to Eq.106 , Eq.123 , Eq.134, Eq.148, Eq. 158 and Eq.159. The equivalent volumes could be obtained from partial regression coefficients which calculated by introduction of the thermodynamic dissociation constant ($Ka°$) from single weak acid titration in these equations and with the use of multiple linear regression analysis (Draper and Smith, 1966 ; Dunteman,1984 ; Johnson and Bhattacharya, 1987 ; Byrkit, 1987) and computer program SPSS/PC$^+$.

3. For the partial regression coefficients determination, ranges of the raw data of two-mixed weak acids titration had to be chosen before being interpreted by the multiple linear regression analysis. The methods used for choosing the ranges of raw data and determination the equivalent volumes of the individual acids could be classified into three methods (Table 1), as followed.

where F   = The statistical value obtained from the analysis of variance. (F - test)

V_I = The initial volume of titrant obtained from comparing V_{iA} and V_{iB}, then the less value was selected.

V_F = The final volume of titrant.

V_{FA} = The volume of titrant which gave pH equal to maximum pH of G plot linearity range of acid A.

V_{FB} = The volume of titrant which gave pH equal to maximum pH of G plot linearity range of acid B.

3.1 Method A:

The raw data of two-mixed weak acids titration would be chosen in the range of V_I to V_F which gave pH equal to maximum pH of Gran plots linearity range of the weaker acid titration (pH_{maxB}). This range of raw data would be interpreted, then Ve_A and Ve_B could be determined from the partial regression coefficients (a_1, a_2), as shown in Figure 4.

3.2 Method B:

3.2.1 For Ve_A determining, the raw data of two-mixed weak acid titration would be chosen in the range of V_I to V_{FA} which gave pH equal to maximum pH of Gran plots linearity range of the stronger

acid titration (pH_{maxA}), as shown in Figure 5. This range of raw data would be interpreted, then Ve_A could be determined from the partial regression coefficient (a_1).

3.2.2 For Ve_B determining, the raw data of two-mixed weak acids titration would be chosen in the range of V_I to V_{FB} which gave pH equal to maximum pH of Gran plots linearity range of the weaker acid titration (pH_{maxB}). This range of raw data would be interpreted, then Ve_B could be determined from the partial regression coefficient (a_2).

3.3 <u>Method C</u> :

The raw data of two-mixed weak acids titration would be chosen in the range of V_I to V_F which gave F value maximum as shown in Figure 6, then Ve_A and Ve_B could be determined from the partial regression coefficients (a_1, a_2).

4. The equivalent volumes of the individual weak acids obtaining from the titration of the two-mixed weak acids mixtures by Method A, B, and C described above were then compared to those obtained from Gran plots of the titration of each single weak acids titrations. To determine whether there was a statistical difference between these results, the student t-test at 95% confidence interval was employed (Devore, and Peck, 1990). Gran plots could be used as the reference method since it had been shown in the former study that there was no

statistical difference between the result obtained from Gran plots and the official method in USP XX (Arttamangkul, 1986).

2.11 The steps to achieve the appropriate experimental conditions.

1. Single weak acid solutions of different initial concentrations were titrated with 0.1 N sodium hydroxide standard solution. The equivalent volume could be determined by extrapolation of linear plot of modified Gran method. The slope of this plot was the thermodynamic dissociation constant (Ka°) at temperature 28 ° C . The equivalent volumes of weak acid could be determined from the intercept. The data range interpreted should be the range of not less than ten points which gave maximum r^2 . The minimum concentration according to this specification was the most probable the minimum initial concentration of this investigation.

2. To study the effect of atmospheric carbon dioxide, the nitrogen gas was passed continuously over the titration solution of single weak acid solution. The equivalent volume obtained from modified Gran methods of this solution was compared to that obtained from modified Gran methods of the single weak acid solution but without nitrogen . Statistical difference was tested by using student t-test at 95% confidence intervals.

3. The precision of potentiometric titration of two weak acid mixtures by multiple linear regression analysis.

Experimental titrations always involve the random errors of measurement of both the pH and the volume of reagent since these errors usually affected on the theoretically attainable precisions of the equivalent volumes obtaining from the modified equation by multiple linear regression analysis. The equation used to evaluate the maximum theoretically error of the equivalent volumes of two weak acid mixtures were illustrated by Eq.191 and Eq.192.

The condition equation used to evaluate the equivalent volumes of weak acid and its mixtures was described by Eq.42 .

The combination of important parameters such as dissociation constant of acid A and acid B (Ka_A, Ka_B), initial concentration ratio (X) of both acid A and acid B, ionization product constant of water (Kw), concentration of titrant used (normality of sodium hydroxide) and initial volume of solution being titrated (Vo), with theoretical coordinates of 30 points along a titration curve were computed from Eq.197. The contemplated operation of an "intelligent" autotitrator was simulated by distributing these points in the following way. The first point always corresponded to $f = 0$ (the start of the titration), and the value of f at each subsequent point was obtained by adding an increment Δf to the last preceding value. The algorithm employed to generate the values of Δf was

$$1. \ f < 1 \qquad \Delta f = 0.004 \qquad\qquad \text{Eq.203}$$

$$2. \ 1 < f < 1 + X \qquad \Delta f = \frac{X}{25}$$

3. stop at $f = 1 + X$

where f = titration parameter = VN/V_OC_{OA}

C_{OA} = initial concentration of weak acid A

C_{OB} = initial concentration of weak acid B

X = concentration ratio = $C_{OB}/C_{OA} = V_{eB}/V_{eA}$

r = dilution parameter = C_{OA}/N

to these definitions, f was equal to 1 at the first equivalent point and to $(1+X)$ at the second.

The value of $[H^+]$ at each point was obtained by solving Eq.197 using Newton-Raphson procedure that was terminated when two successive approximations differed by 0.001% or less, and was then converted into a value of the pH after that. In making this conversion, the value of the apparent single ion activity coefficient γ_{H^+} was assumed to be 1. Since γ_{H^+} was taken to be a constant, experiments based on the results of the calculations had to ,of course, be conducted in such a way as to minimize or eliminate its variation. Adding a swamping concentration of a neutral electrolyte would serve that purpose and would also minimize variations of the liquid-junction potential which also affected γ_{H^+}.

The resulting data pairs were combined with a general program (Macro Lotus) for estimating the variances of the parameters in multiple linear regression analysis. That program included provision for errors in measurements of the independent variable as well as the dependent one. It applied an increment equal $5\sigma pH$, where σpH was the value assigned to the standard deviation of a measurement of pH, to the pH value for each point in turn. For each of the perturbed sets of data thus produced, it found the best values of the various parameters Ve_A, Ve_B by multiple linear regression analysis, stored the resulting value of $\Delta Ve_A/\Delta pH$ and $\Delta Ve_B/\Delta pH$ as estimators of the partial derivatives $\partial Ve_A/\partial pH$ and $\partial Ve_B/\partial pH$, respectively at the point under consideration. Then, restored the pH value that was altered, and went on the next point. After effecting the fit and restoring the original pH value at the last point, it obtained estimates of the derivatives $\partial Ve_A/\partial V$ and $\partial Ve_B/\partial V$ where V was the volume of base, in a similar way. Finally it combined the values of the derivatives to yield estimates of the variances of the parameters which were obtained from Eq. 191.

In synthesizing the "data", it was always assumed that N = 0.1 normal, $Kw = 1.2846 \times 10^{-14}$ mol^2 dm^{-6} at 28 degree cencius and $\gamma_{H^+} = 1$. The values of pKa_A were varied from 4 to 8, pKa_B from 5 to 9, ΔpKa from 5 to 1 , initial concentration ratios from 0.05 to 40, σ_{pH} and σ_V were usually taken to be 0.002 pH and 0.005 , respectively. These standard errors of measurement were slightly, but in modern practice not excessively, optimistic, and it was shown in the following chapter how changes of their values affected the standard errors of

parameters. These equations contained two parameters: V_{eA} and V_{eB}. Eq.192 could also use to evaluate the precision due to combination of the standard deviation of pH observed and volume of titrant used.