CHAPTER III

EXPERIMENTS

Equipments :

    1. pH meter (ϕ 50 Beckman)

    2. Combined glass electrode (39539 S302B Beckman)

    3. 655 Multidosimat (Metrohm Herisau)

    4. Automatic titrator and exchange unit model 3005(301)

    5. Magnetic stirror E 649 (Metrohm Herisau)

Materials (Budavari , ed., 1989) :

1. <u>Neutral weak acids</u>

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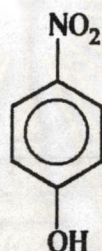

COOH

Empirical formular : $C_7H_6O_2$

Molecular weight : 122.12

pKa (28 °C) (Supawadee , 1993) : 4.1

Description : monoclinic tablets, plates, leaflets

Solubility   : one gram dissolves in 2.3 ml ether , 3 ml acetone , 10 ml benzene , 30 ml carbon disulfide , 23 ml oil of terpentine , 250 ml water (25 $^{\circ}$C) and 230 ml water (30 $^{\circ}$C).

1.2 p-Nitrophenol , AR (Fluka Chemica, Lot # 73560)

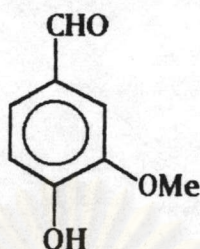
Empirical formular   : $C_6H_5NO_3$

Molecular weight   :   139.11

Description   :   colorless to slightly yellow , odorless crystals , sweetish, then burning taste

pKa (28 $^{\circ}$C) (Supawadee , 1993) :   7.0

Solubility   :   Moderately sol. in cold water , freely in alcohol , chloroform , ether , also sol. in solution of fixed alkali hydroxide and carbonates.

1.3 <u>Vanillin</u> , AR (Fluka Chemica, Lot # 94750)

Empirical formular : $C_8H_8O_3$

Molecular weight : 152.15

Description : white or very slightly yellow needles ,
pleasant aromatic vanilla odor.

pKa (20 °C) (Jackson , Moss , and Widdop ,ed., 1986) : 7.4

Solubility : 1 gram dissolves in 100 ml water , about
20 ml glycerol , freely sol. in alcohol ,
ether , chloroform , carbon disulfide.

1.4 <u>Salicylamide</u>, AR (E.Merck, Lot # 535850)
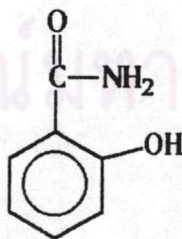
Empirical formular : $C_7H_7NO_2$
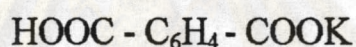
Molecular weight : 137.14

Description   : white or slightly pink, crystalline powder, somewhat bitter taste.

pKa (25 $^\circ$C)   : 8.1

Solubility (Florey , ed.,1984) : one gram dissolves in 500 ml water , 15 ml alcohol , 35 ml ether , and 100 ml chloroform.

2. Ionized weak acids

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

$$HOOC - C_6H_4 - COOK$$

Empirical formular : $C_8H_5KO_4$
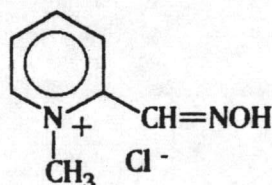
Molecular weight : 204.23

Description   : orthorhombic crystals , stable in air

pKa (28 $^\circ$C) (Supawadee , 1993) : 5.0

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

2.2 Pralidoxime chloride , (Sigma , Lot # 60HO445)
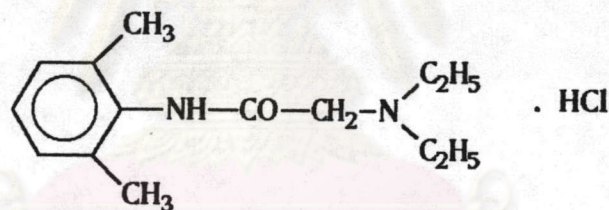
Empirical formular : $C_7H_9N_2O \cdot Cl$

Molecular weight : 172.63

Description (Florey, ed., 1988) : crystalline , odorless , stable in air at temp. below 100 °C , white to pale yellow.

pKa (25 °C) : 7.8 - 8

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

2.3 <u>Lidocaine hydrochloride</u> , (Sigma, Lot # 62HO959)

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

Molecular weight : 270.82

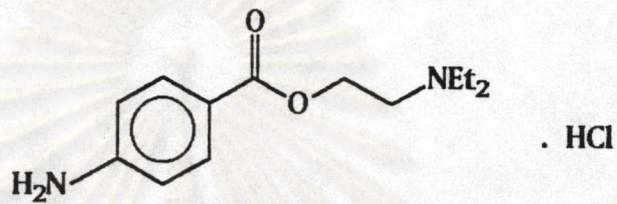Description (Florey,ed.,1985): both the base and hydrochloride are white odorless substance.

pKa (25 °C) : 7.8

Solubility(25°C) : HCl salt : 0.68 Gm in 1 ml water

free base : 0.004 Gm in 1 ml water

0.76 Gm in 1 ml 95% ethanol

0.79 Gm in 1 ml chloroform

0.12 Gm in 1 ml n-hexane

2.4 Procaine hydrochloride, AR

Empirical formular : $C_{13}H_{20}N_2O_2 \cdot HCl$

Molecular weight : 272.8

Description : colorless crystals or a white crystalline powder, odorless

pKa (25 °C) : 9.0

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

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

Solvent and Other Reagents :

1. Sodium hydroxide , AR (E.Merck, Lot # 327K19742898)
2. Potassium chloride , AR (E.Merck, Lot # 208TA253636)
3. Distilled water (Freshly prepared)
4. Electrode filling and storage solution , 4 M KCl saturated with AgCl , (Beckman, Lot # S306301)
5. Standard buffer solution pH 4 (Beckman,Lot # S308171)
6. Standard buffer solution pH 7 (Beckman,Lot # S309141)

Preparation of sodium hydroxide standard solution 0.1 N

Sodium hydroxide was dissolved in an equal weight of water and allowed to stand overnight. Taking precaution to avoid absorption of carbon dioxide. Siphon off or decant the clear supernatant liquid and dilute 5.5 ml with carbon dioxide-free water to produce 1000 ml . (Recommendation of the Medicine Commision , 1988)

Standardization of sodium hydroxide solution

As certain it exact concentration immediately before use by titrating with it a solution of 50 mg of potassium biphthalate , previously crushed lightly and dried at 120 °C for 2 hours , in 50 ml of carbon dioxide-free water , and titrated with sodium hydroxide standard solution. Calculate the normality of solution which each ml of 1.000 N sodium hydroxide is equivalence to 204.22 mg of potassium biphthalate.

Preparation of weak acidic compounds (0.005 M) in 0.1 M KCl

Potassium chloride was weighed and dissolved in the distilled deionized water , and dilute with the same solvent to give a final concentration about 0.1 M . Each weak acidic compound was accurately weighed in suitible quantities (according to its molecular weight) to produce about 0.01 M solution. They were dissolved in the solution of 0.1 M potassium chloride in order to control the ionic strength of the solution through all step of the titration. Transfer the weak acidic compound solution (about 0.01 M in 0.1 M KCl) 25.0 ml and 0.1 M KCl 25.0 ml by transferring pipette to a 100 ml beacker. The final concentration of weak acidic compound solution in 0.1 M KCl was about 0.005 M .

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

Transfer each 25.0 ml of two weak acidic compound solutions which their concentration were about 0.01 M to a 100 ml beaker to produce the final solution which the concentration of each weak acid was about 0.005 M in 0.1M KCl .

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

Fifty milliliters of the pipetted solutions were titrated with 0.1 N sodium hydroxide standard solution. The glass electrode which was calibrated before each titrations by reference to standard buffer solutions (pH 4 and pH 7 at 28 °C) was submerged into titrated solution for 5 minutes prior to commencement of titration to assure that the electrode was in equilibrium with titrated solution. The beaker was

placed on the magnetic stirror. The sample solution was stirred after each addition of titrant and the value of pH was measured after the stirror off. The precaution was not to rinse the side of the beaker with distilled water because of the importance of knowing the exact solution volume at all times. From the titration of the single weak acid solution and two-mixed weak acid solution , the values of pH at each volume of titrant added were measured. Each sample solution was titrated five replicates. The raw data of the experiments would be obtained.

The steps of analysing data for determining the equivalence volumes

1. From the raw data obtained from the titration of each single weak acid solution , the equivalence volume could be determined by these methods below.

1.1 Titration curve : the end point could be located on the steeply rising portion of the curve.

1.2 G plot : the slope of this plot according to Eq.9 and Eq. 21 (page 6 and 9) was the dissociation constant (Ka) at ionic strength 0.1 M (KCl) and temperature 28 °C. The equivalence volumes of each weak acid could be determined from the intercept. The data range interpreted was the range which gave maximum r^2 (see Figure 1 and 2).

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.

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.

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

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

N_1 = the numbers of raw data within G plot linearity
range of acid A.

N_2 = the numbers of raw data within G plot linearity
range of acid B.

2. From the raw data obtained from the titration of two-mixed
weak acid solution , the equivalence 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.69 , Eq.81 , Eq.88 , Eq.91 , Eq.92 or
Eq.93 . The multiple linear regression analysis (Draper and Smith , 1966
; Dunteman , 1984 ; Johnson and Bhattacharya, 1987 ; Byrkit, 1987) and
program computer SPSS/PC[+] were used in order to solve these equations.
The partial regression coefficients could be determined. From the values
of partial regression coefficients , the equivalence volumes could be
obtained.

3. For the partial regression coefficients determining , ranges of
the raw data of two-mixed weak acids titration would be chosen before
being interpreted by the multiple linear regression analysis. The methods
used for choosing the ranges of raw data and determination the

equivalence volumes of the individual acids could be classified into 5 methods (see 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 Vi_A and Vi_B , 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 <u>Method A</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 (see Figure 3) , then Ve_A and Ve_B could be determined from the partial regression coefficients (a_1, a_2).

3.2 <u>Method B</u> : 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 G plot 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 .

3.3 <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 the numbers of data points equal to $N_1 + N_2$. 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 5.

3.4 <u>Method D</u> :

3.4.1 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 G plot linearity range of the stronger acid titration (pH_{maxA}) , as shown in Figure 6.   This range of raw data would be interpreted , then Ve_A could be determined from the partial regression coefficient (a_1).

3.4.2   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 G plot 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).

3.5 <u>Method E</u> : In this method the ranges of raw data interpreted were the same as Method D , but

3.5.1   For Ve_A determining ,   the equivalence volume of the weaker acid (Ve_B) obtained from Method D was substituted into the multiple linear equation ($y = a_1x_1 + a_2x_2$ according to Eq. 69,81, 88 , 91 , 92 and 93) , then the equation would be rearranged to a simple linear equation ($y - a_2x_2 = a_1x_1$) and Ve_A could be determined from the partial regression coefficient (a_1) .

3.5.2 For Ve_B determining , the equivalence volume of the stronger acid (Ve_A) obtained from Method D was substituted into the multiple linear equation in the same manner as Ve_A determining and Ve_B could be determined from the partial regression coefficient (a_2) of the simple linear equation , ($y - a_1x_1 = a_2x_2$) .

4. The equivalence volumes of the individual weak acids obtained from the titration of the two-mixed weak acids mixtures by Method A , B , C , D , and E described above were then compared to those obtained from G plots of the titration of each single weak acids titrations to determined whether there was a statistical difference between these results. The student t-test at 95% confidence interval was employed (Devore ,and Peck , 1990). G plot 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 G plot and the official method in USP XX (Seksiri Arttamangkul , 1986).