

## CHAPTER IV

### RESULT AND DISCUSSION

The computer curve fitting program , SPSS/PC<sup>+</sup>, was used for interpreting the data. The feasibility of this program was proved theoretically by the methods described in previous chapter . The results were summarized in Table 2 . This table shown the comparison between the theoretically equivalent volumes (  $V_{eA}$  and  $V_{eB}$  ) and the calculated equivalent volumes (  $V_{eA}$  and  $V_{eB}$  ) obtained from multiple linear regression analysis . It could be found that this program , SPSS/PC<sup>+</sup>, was suitable in solving the modified equation for equivalent volume determination of two weak acid mixture which had difference in pKa value and initial concentration ratios . From the results of these simulated data which  $\Delta pK_a$  value was in the range of 0.2 to 5 and the initial concentration ratios was in the range of  $2 \times 10^{-4}$  to  $5 \times 10^3$  , the accurate result could be obtained.

The accuracy of potentiometric titration of two weak acid mixtures was studied. The seven acidic compounds selected were weak acid with pKa value between 4-9 and could dissolve in 0.1 M KCl solution with no precipitate during the course of titration. These compounds were benzoic acid, pivalic acid, *p*-nitrophenol, pralidoxime

chloride, lidocaine hydrochloride, boric acid, and procaine hydrochloride which their pKa values were shown in Table 3. The scope of this experiment was to study the binary mixtures of weak acids which had the difference in pKa values ( $\Delta pK_a$ ) and in the initial concentration ratios. For matching the pairs of them, they were arranged in random form in order to reduce the bias. These weak acid mixtures and their pKa values were presented in Table 4.

The binary mixtures of weak acidic compounds were classified into three categories, as followed.

1. The mixtures between two neutral weak acids.
2. The mixtures between neutral weak acid and ionized weak acid.
3. The mixtures between two ionized weak acids.

The results obtained from the multiple linear regression analysis of the titrations of weak acid mixture were compared to those obtained from the reference method (Gran plots or G plot) of the titration of each single acid titrations. To determine whether there was a statistical difference between these results, the student t-test at 95% confidence interval was employed. Gran plots (G plot) could be used as the reference method, since it had been shown in the previous study (Arttamangkul, 1986) that there was no statistical difference between the result obtained from G plot and the official method in USP XX (non-aqueous titration).

The results obtained from this study could be discussed as followed.

## I. The titration of two neutral weak acids mixtures

The neutral weak acid mixtures in this study were

### 1.1 Benzoic acid and pivalic acid mixture

$$(X = 1, 5, 10, 15, 0.5)$$

### 1.2 Benzoic acid and *p*-nitrophenol mixture

$$(X = 1, 5, 10, 0.5)$$

### 1.3 Benzoic acid and boric acid mixture

$$(X = 1, 5, 10, 15, 0.5)$$

### 1.4 Pivalic acid and *p*-nitrophenol mixture

$$(X = 1, 5, 10, 0.2, 0.1)$$

### 1.5 Pivalic acid and boric acid mixture

$$(X = 1, 5, 10, 15, 0.2, 0.1)$$

### 1.6 *p*-nitrophenol and boric acid mixture

$$(X = 1, 5, 10, 15, 0.2, 0.1)$$

where X = the initial concentration ratio of acid mixtures

$$= V_{eB}/V_{eA}$$

#### 1.1 Benzoic acid and pivalic acid mixture

The dissociation constants of these compounds as determined by Gran plots were  $8.33 \times 10^{-5}$  ( $pK_a = 4.08$ ) for benzoic acid and  $9.77 \times 10^{-6}$  ( $pK_a = 5.01$ ) for pivalic acid. The difference between  $pK_a$  values of these acids was about 0.93. From the titration of each weak acid solutions, the equivalent volumes could be determined from the titration curves and Gran plots as shown in Figure 7 to 18. The titration curves of these acids

mixture were shown in Figure 53 to 57 with data presented in Table 5 to 14.

It could be seen that the equivalent volumes of the individual weak acids obtained from the titration of these acids mixture, which the initial concentration ratios ( $X$ ) were 1, 5, 10, 0.5 were statistical indifference from those obtained from Gran plots of each single weak acid titrations when Method B was used for choosing the range of raw data. However, there were statistical difference when initial concentration ratios ( $X$ ) was changed to 15.

When Method A and C were used instead of Method B, it was found that there were statistical difference between the equivalent volumes of each weak acids obtained from the acid mixture titration and those obtained from Gran plots of each single acid titrations as shown in Table 119 for all initial concentration ratios ( $X = 1, 5, 10, 15, 0.5$ ).

Thus, the modified equation (Eq.106) and Method A and Method C used for choosing the range of raw data in this study could not be used to determine accurate and reproducible equivalent volumes of the individual weak acids from these acids mixture titration. Nevertheless, Method B could be used successfully with Eq.106 when initial concentration ratios were 1, 5, 10, 0.5 but failed when initial concentration ratios was 15.



## 1.2 Benzoic acid and *p*-nitrophenol mixture

The dissociation constants of these compounds as determined by Gran plots were  $8.33 \times 10^{-5}$  ( $pK_a = 4.08$ ) for benzoic acid and  $8.97 \times 10^{-8}$  ( $pK_a = 7.05$ ) for *p*-nitrophenol. The difference between  $pK_a$  values of these acids was about 2.97. From the titration of each weak acid solutions, the equivalent volumes could be determined from the titration curves and Gran plots as shown in Figure 7 to 10 and 19 to 24. The titration curves of these acids mixture were shown in Figure 58 to 61 with the data presented in Table 15 to 22.

The equivalent volumes of the individual acids obtained from the titration of these acids mixture whose initial concentration ratios ( $X$ ) were 1, 5, 10 and 0.5 were statistical indifference from those obtained from Gran plots of each single weak acid titrations when Method B was used for choosing the range of raw data.

When Method A and C were used for choosing the raw data range there were statistical indifference between the equivalent volumes of each weak acid obtained from the acid mixture titration and those obtained from Gran plots of each single acid titrations when the initial concentration ratios were 1 or 0.5. In contrast, it showed statistical difference when the initial concentration ratios were 5 and 10 as presented in Table 119.

The correlation of the results obtained from Eq.106 was the same as described earlier with the mixture of benzoic acid and pivalic acid.

### 1.3 Benzoic acid and boric acid mixture

The dissociation constants of these compounds as determined by Gran plots were  $8.33 \times 10^{-5}$  ( $pK_a = 4.08$ ) for benzoic acid and  $7.87 \times 10^{-10}$  ( $pK_a = 9.10$ ) for boric acid. The difference between  $pK_a$  values of these acids was about 5.02. From the titration of each weak acid solutions, the equivalent volumes could be determined from the titration curves and Gran plots as shown in Figure 7 to 10 and 39 to 48. The titration curves of these acids mixture were shown in Figure 62 to 66 with the data presented in Table 23 to 32.

It could be seen that there were statistical indifference in the equivalent volumes of the individual weak acids obtained from the titration of these acids mixture, which the initial concentration ratios (X) were 1, 5, 10, and 0.5 when Method B was used for choosing the range of raw data except with the initial concentration ratios of 15.

In case of Method A and C, they showed statistical difference for initial concentration ratios 5, 10 and 15. On the contrary, they showed statistical indifference for initial concentration ratios 1 and 0.5 (Table 119).

The correlation of the results obtained from Eq.106 was the same as described earlier with the mixture of benzoic acid and pivalic acid.

#### 1.4 Pivalic acid and *p*-nitrophenol mixture

The dissociation constants of these compounds as determined by Gran plots were  $9.77 \times 10^{-6}$  ( $pK_a = 5.01$ ) for pivalic acid and  $8.97 \times 10^{-8}$  ( $pK_a = 7.05$ ) for *p*-nitrophenol. The difference between  $pK_a$  values of these acids was 2.04. From the titration of each single weak acid solutions, the equivalent volumes could be determined from the titration curves and Gran plots as shown in Figure 11 to 24. The titration curves of these acids mixture were shown in Figure 67 to 71 with the data presented in Table 33 to 42.

It could be seen that there were statistical indifference in the equivalent volumes of the individual weak acids obtained from the titration of the acid mixtures whose initial concentration ratios ( $X$ ) were 1, 5, 10, and 0.2 when Method B was used for choosing the range of raw data except with the initial concentration ratios of 0.1.

In case of Method A and C, they showed statistical difference for all concentration ratios studied here ( $X = 1, 5, 10, 0.2$  and  $0.1$ ) as shown in Table 119.

The correlation of the results obtained from Eq.106 was the same as described earlier with the mixture of benzoic and pivalic acid.

### 1.5 Pivalic acid and boric acid mixture

The dissociation constants of these compounds as determined by Gran plots were  $9.77 \times 10^{-6}$  ( $pK_a = 5.01$ ) for pivalic acid and  $7.87 \times 10^{-10}$  ( $pK_a = 9.10$ ) for boric acid. The difference between  $pK_a$  values of these acids was 4.09. From the titration of each weak acid solutions, the equivalent volumes could be determined from the titration curves and Gran plots as shown in Figure 11 to 18 and 39 to 48. The titration curves of these acids mixture were shown in Figure 72 to 77 with the data presented in Table 43 to 54.

It could be seen that there were statistical indifference in the equivalent volumes of the individual weak acids obtained from the titration of these acids mixture whose initial concentration ratios ( $X$ ) were 1, 5, 10 and 0.2 from those obtained from Gran plots of each single weak acid titrations when Method B was used for choosing the range of raw data except with the concentration ratios of 15 and 0.1.

In case of Method A and C, they showed statistical difference for concentration ratios 5, 10, 15, 0.2 and 0.1 except with the initial concentration ratios of 1 (Table 119).

The correlation of the results obtained from Eq.106 was the same as described earlier with the mixture of benzoic acid and pivalic acid.

### 1.6 *p*-nitrophenol and boric acid mixture

The dissociation constants of these compounds as determined by Gran plots were  $8.97 \times 10^{-8}$  ( $pK_a = 7.05$ ) for *p*-nitrophenol and  $7.87 \times 10^{-10}$  ( $pK_a = 9.10$ ) for boric acid. The difference between  $pK_a$  values of these acids was 2.05. From the titration of each weak acid solutions, the equivalent volumes could be determined from the titration curves and Gran plots as shown in Figure 19 to 24 and 39 to 48. The titration curves of these acids mixture were shown in Figure 78 to 83 with the data presented in Table 55 to 66.

There were statistical indifference in the equivalent volumes of the individual acids obtained from the titration of the acid mixtures, whose the initial concentration ratios ( $X$ ) were 1, 5, 10 and 0.2 when Method B was used for choosing the range of raw data except with the ratio of 15 and 0.1.

In case of Method A and C, they demonstrated the statistical difference for all the initial concentration ratios studied here ( $X = 1, 5, 10, 15, 0.2$  and  $0.1$ ) as shown in Table 119.

Thus, the modified equation (Eq.106) when being used with Method A and Method C could not be applied for choosing the range of raw data to determine accurate and reproducible equivalent volumes of the individual weak acids from these acids mixture titration.

From the results of this group (the mixtures of two neutral weak acids), it could be concluded that Method B was the best method for choosing titration data range to determine equivalent volumes of each weak acids. The pairs of weak acids mixture which their equivalent volumes could be determined by the multiple linear regression analysis and computer program, SPSS/PC<sup>+</sup> were the acid mixtures which the initial concentration ratios were not equal to 15 and 0.1. We call these two initial concentration ratios as the upper initial concentration ratios limit and the lower initial concentration ratios limit. Method A and Method C also could be used to determine equivalent volumes of each weak acids if the acid mixtures had the difference between  $\Delta pK_a$  value much more than 2 ( $\Delta pK_a = 2.97, 4.09$  and  $5.02$  as presented in this experiment) and the initial concentration ratios of the acid mixtures were  $X = 1$  or  $X = 0.5$ .

## II. The titration of the mixtures of neutral weak acid and ionized weak acid.

The neutral weak acid and ionized weak acid mixtures in this study were

### 2.1 Benzoic acid and pralidoxime chloride mixture

( $X = 1, 5, 10, 15, 0.5$ )

### 2.2 Pivalic acid and pralidoxime chloride mixture

( $X = 1, 5, 10, 15, 0.2, 0.1$ )

### 2.3 *p*-nitrophenol and pralidoxime chloride mixture

( $X = 1, 5, 10, 15, 0.2, 0.1$ )

## 2.4 Pralidoxime chloride and boric acid mixture

$$(X = 1, 5, 10, 0.2, 0.1)$$

where  $X$  = the initial concentration ratio of acid mixtures

$$= V_{eB}/V_{eA}$$

### 2.1 Benzoic acid and pralidoxime chloride mixture

The dissociation constants of these compounds as determined by Gran plots were  $8.33 \times 10^{-5}$  ( $pK_a = 4.08$ ) for benzoic acid and  $1.10 \times 10^{-8}$  ( $pK_a = 7.96$ ) for pralidoxime chloride. The difference between  $pK_a$  values of these acids was 3.88. From the titration of each weak acid solutions, the equivalent volumes could be determined from the titration curves and G plot as shown in Figure 7 to 10 and 25 to 34. The titration curves of these acids mixture were shown in Figure 84 to 88 with the data presented in table 67 to 76.

It could be seen that there were statistical indifference between the equivalent volumes of the individual acids obtained from the titration of the acid mixtures whose the initial concentration ratios were 1, 5, 10 and 0.5 from those obtained from Gran plots of each single weak acid titrations when Method B was used for choosing raw data titration range except with the concentration ratios ( $X$ ) of 15 that showed statistical difference.

In case of Method A and C, they both demonstrated statistical indifference with the initial concentration ratios 1 and 0.5. However,

statistical difference was observed when the initial concentration ratios changed to 5, 10 and 15.

The correlation of the results obtained from Eq.106 was the same as described earlier with the mixture of benzoic acid and pivalic acid.

## 2.2 Pivalic acid and pralidoxime chloride mixture

The dissociation constants of these compounds as determined by Gran plots were  $9.77 \times 10^{-6}$  ( $pK_a = 5.01$ ) for pivalic acid and  $1.10 \times 10^{-8}$  ( $pK_a = 7.96$ ) for pralidoxime chloride. The difference between their  $pK_a$  values was 2.95. From the titration of each single weak acid solutions, the equivalent volumes could be determined from the titration curves and Gran plots as shown in Figure 11 to 18 and 25 to 34. The titration curves of these acids mixture were shown in Figure 89 to 94 with the data presented in Table 76 to 88.

There were statistical indifference in the equivalent volumes of each weak acid obtained from the titration of the acid mixtures whose the initial concentration ratios (X) of the acid mixtures were 1, 5, 10 and 0.2 when Method B was used for choosing the raw data range except with the initial concentration ratios of 15 and 0.1.

In case of Method A and Method C, they demonstrated statistical difference of most concentration ratios studied here (X = 5, 10, 15, 0.2 and 0.1) except the concentration ratio 1 (Table 119).

The results of these acids mixture titrations were the same manner as previously described for the titration of benzoic acid and pivalic acid mixture.

### 2.3 *p*-nitrophenol and pralidoxime chloride mixture

The dissociation constants of these compounds as determined by Gran plots were  $8.97 \times 10^{-8}$  ( $pK_a = 7.05$ ) for *p*-nitrophenol and  $1.10 \times 10^{-8}$  ( $pK_a = 7.96$ ) for pralidoxime chloride. The difference between  $pK_a$  values of these weak acids was 0.91. From the titration of each single weak acid solutions, the equivalent volumes could be determined from the titration curves and Gran plots as shown in Figure 19 to 34. The titration curves of these acids mixture were shown in Figure 95 to 100 with the data presented in table 89 to 100.

It could be seen that there were statistical indifference between the equivalent volumes of the individual acid obtained from the titration of the acid mixtures whose the initial concentration ratios were 1, 5, 10 and 0.2 from those obtained from Gran plots of each single weak acid titrations when Method B was used for choosing the range of raw data except with the concentration ratios of 15 and 0.1 showed statistical difference.

In case of Method A and C, they demonstrated statistical difference for all the initial concentration ratios (Table 119).

The results of these acids mixture titrations were the same manner as previously described for the titration of *p*-nitrophenol and boric acid mixture.

#### 2.4 Pralidoxime chloride and boric acid mixture

The dissociation constants of these compounds as determined by Gran plots were  $1.10 \times 10^{-8}$  ( $pK_a = 7.96$ ) for pralidoxime chloride and  $7.87 \times 10^{-10}$  ( $pK_a = 9.10$ ) for boric acid. The difference between  $pK_a$  values of these acids was 1.14. From the titration of each single weak acid solutions, the equivalent volumes could be determined from the titration curves and G plots as shown in Figure 25 to 34 and 39 to 48. The titration curves of these acids mixture were shown in Figure 101 to 106 with the data presented in Table 101 to 112.

It could be seen that the equivalent volumes of the individual weak acids obtained from the titration of this mixture which the initial concentration ratios (X) were 1 and 0.2 were statistical indifference from those obtained from Gran plots of each single weak acid titrations when Method B was used for choosing the range of raw data except with the concentration ratios of 5, 10 and 0.1.

In case of Method A and C, they demonstrated statistical difference for all the initial concentration ratios (Table 119).

As a consequence, the modified equation 123 and Method A and Method C could not be used to determine accurate and reproducible

equivalent volumes of the individual weak acids from these acids mixture titration.

From the results of this group (the mixtures of neutral weak acid and ionized weak acid), it could be concluded that Method B was the best method for choosing titration data range to determine equivalent volumes of each weak acids. The pairs of weak acid mixtures which their equivalent volumes could be determined by the multiple linear regression analysis and computer program, SPSS/PC<sup>+</sup> were the acid mixtures which the initial concentration ratios were not equals to 15 and 0.1, the same conclusion as the results from the titration of two neutral weak acids. Exception was found with the titration of pralidoxime chloride and boric acid mixtures which Method B could not be used for determining accurate equivalent volumes of the individual weak acids from this binary acids mixture titration even if the initial concentration ratios were 5, 10 and 0.1.

Method A and Method C also could be used to determine equivalent volumes of each weak acids if the acid mixtures had the difference between pKa value much more than 2 ( $\Delta pK_a = 2.95$  and  $3.88$  as in this experiment) and the initial concentration ratios of the acid mixtures were  $X = 1$  or  $X = 0.5$ . This was the same conclusion as the results from the titration of two neutral weak acids.

### III. The titration of two ionized weak acid mixtures

The two ionized weak acids mixtures in this study was lidocaine hydrochloride and procaine hydrochloride mixture ( $X = 1, 5, 0.5$ )

where  $X =$  the initial concentration ratios of the acid mixtures

$$= V_{eB}/V_{eA}$$

#### 3.1 Lidocaine hydrochloride and procaine hydrochloride mixture.

The dissociation constants of these compounds as determined by Gran plots were  $1.18 \times 10^{-8}$  ( $pK_a = 7.93$ ) for lidocaine hydrochloride and  $8.93 \times 10^{-10}$  ( $pK_a = 9.05$ ) for procaine hydrochloride. The difference between  $pK_a$  values of these acids was 1.12. From the titration of each weak acid solutions, the equivalent volumes could be determined from the titration curves and Gran plots as shown in Figure 35 to 38 and 49 to 52. The titration curves of these acids mixture were shown in Figure 107 to 109 with the data presented in Table 113 to 118.

It could be seen that there were statistical indifference in the equivalent volumes of the individual acids obtained from the titrations of the acid mixtures whose initial concentration ratios were 1 and 0.5 when Methods B was used for choosing the range of raw data.

In case of Method A and C, which applied into the modified equation 148, could not be used for determining accurate equivalent

volumes of the individual weak acids from this binary acids mixture titration of all the initial concentration ratios studied here ( $X = 1, 5, 0.5$ ) as shown in Table 119.

For the titration of this acid mixtures which the initial concentration ratios was  $X = 1$  and  $0.5$ , the results from these acid mixture titrations corresponded to those obtained from the acid mixture titration of pralidoxime chloride and boric acid mixture except when the initial concentration ratios  $X = 5$ , the precipitation occurred during the course of titrations; therefore, the modified equation 148 could not be used to determine the equivalent volume of each single weak acid from the binary weak acid mixtures.

From the results of this acid mixture titration, it could be concluded that Method B was the best method for choosing titration data range to determine equivalent volumes of each weak acid from the acid mixtures which the initial concentration ratios were  $1$  and  $0.5$  and the difference between the  $pK_a$  values of each weak acids were  $1.12$ . Method A and Method C could not be used to determine equivalent volumes of each weak acid in the acid mixtures of lidocaine hydrochloride and procaine hydrochloride.

From the results of these experiments (Table 119), it would be concluded that there were many factors which affected to the accuracy and precision of the equivalent volumes obtained from the titration of binary acid mixtures; i.e., the titration data range applied for analysis, the

difference between  $pK_a$  values of weak acids, the initial concentration ratios of the acid mixtures, the ionic strength of solution during the course of titration.

**Factors affecting the accuracy and precision of the equivalent volumes of the individual weak acid obtained from the titration of binary acid mixtures with different  $\Delta pK_a$  and different initial concentration ratios of the acid mixtures.**

### **I. The effect of experimental condition .**

In using Gran plots, there were several factors affecting the accuracy and precision of the determination of equivalent volumes.

The multiple linear equation used in this study was derived on the basis of mass balance, charge balance and equilibrium equation which was the same manner as the derivation of the equations in Gran methods of single acid titration. Thus, those factors affect errors in acid base titration using Gran plots should also affect errors in acid base titration using multiple linear regression analysis method. The error sources might be described as followed :

#### **1.1. The effect of experimental conditions.**

The experimental conditions used for this study should be optimized for several reasons (Akimoto, Hanakuma and Hozumi; 1987) as followed:

1.1.1 Complications arising from the chemistry of the reaction were minimal; therefore, titration errors related to the way in which the data were analyzed could be highlighted.

1.1.2 The conditions were very nearly the same in titration of five replicately samples.

1.1.3 Accurate measurements of the end point of each single acid titration could be obtained from Gran method and used as a reference for multiple linear method studied here.

The appropriate condition investigated could be minimize the error in the study of acid mixture titration.

## 1.2. Temperature effect

Variation of temperature affects various parameters; e.g. , liquid junction potential, activity coefficients, dissociation constant; however, nearly ineffectiveness in case of thermodynamic dissociation constant (Barry, Meites and Campbell; 1974). Thus, carefully control of temperature in variation of  $\pm 0.5$  °C seem to be necessary (Barry and Meites; 1974).

## 1.3. The effect of carbon dioxide

Since atmospheric carbon dioxide might be dissolved in the titration solution and caused an error in equivalent volume detection, as well as, carbonate, an impurity in the sodium hydroxide preparation, could also created slow and less precise electrode response in poorly buffered solution; thus, nitrogen gas was passed over the solution during

titration in order to prevent possible dissolution of atmospheric carbon dioxide.

There were also many factors which affecting the accuracy and precision of the equivalent volume evaluation in the two weak acid mixtures. Then, the more scrupulous care was taken in calibrating and cleaning the glassware, in two-point standardization of the pH meter before each titration, in avoiding electronic circuit drift during titration by choosing the appropriate apparatus and environment system. Carefully control in experiment could establish the accurate result in evaluating the equivalent volumes of each weak acid in the titration of two weak acid mixtures.

## **II. The effect of titration data range applied for analysis**

From Table 119,120 and 121, it was concluded that the titration data range of acid mixture titration applied to the multiple linear regression analysis, affected to the interpretation of the equivalent volumes of the individual acids obtained. The appropriate method used for choosing titration data range was Method B. For this method, the accurate results would be obtained if the initial concentration ratios of the acid mixtures were not 15 and 0.1 for those acid mixtures with any difference  $\Delta pK_a$  except the acid mixtures of pralidoxime chloride - boric acid which their  $pK_a$  values were 8 and 9 ( $\Delta pK_a = 1$ ). For this acid mixtures (pralidoxime chloride - boric acid mixtures), the accurate and

reproducible results could be obtained by using Method B choosing the raw data range only when the initial concentration ratios were 1 and 0.2 .

For Method A and Method C, the accurate results would be obtained if the difference between pKa values of weak acids was much more than 2 (2.95, 2.97, 3.88, 4.09, 5.02 as in this experiment) and the initial concentration ratios were 1 or 0.5. The results obtained from each method could be discussed as followed.

2.1 Method A : For this method, the raw data of two-mixed weak acids titration would be chosen in the range of  $V_1$  to  $V_F$  which gave pH equal to maximum pH of G plot range of the weaker acid titration, then  $V_{eA}$  and  $V_{eB}$  were determined from the partial regression coefficients ( $a_1$  and  $a_2$ ) of the multiple linear equation. The pairs of mixed weak acids which had no statistical difference between the equivalent volumes obtained from multiple regression analysis of two-mixed weak acids titration and G plot of each single acids titrations were the acid mixtures which their difference between pKa values of weak acids was much more than 2 and their initial concentration ratios were 1 or 0.5 ( Table 119).

As considered to the other pairs of acids mixtures, this method could not yield accurate and reproducible results of  $V_{eA}$  determination when compared to those obtained by Method B ( Table 120). In this case, it might be due to the final part of data range chosen by this method were the data points around and after the equivalent points of acid A (stronger

acids). Thus, errors in  $V_{eA}$  determining might be happen. The reasons would be described, as followed.

The multiple linear equation used in this study was derived on the basis of mass balance, charge balance and equilibrium equation which was the same manner as the derivation of the equations in Gran method of single acid titration. For Gran plots of weak acid titration, there were deviations from linearity which affected to the accuracy and precision of results obtained (Macca and Bombi, 1989, Prachasitthisak, 1996) : that deviation occurred in the initial part and before the equivalent point. Thus, the titration data range of two-mixed weak acids used for determining  $V_{eA}$  and  $V_{eB}$  should be within the linearity range of Gran plots of each single acid titrations.

Deviations from the linear ideal behavior of Gran plots could be due to the unreacted amount of the relevant reactant be not equal to its own stoichiometric excess, because the reaction was incomplete (Macca and Bombi, 1989). Such deviations occurs obviously in the system of the acid mixtures which their  $\Delta pK_a$  was small so that in the first buffer region of titration, the weaker acid could be ionized and reacted with the titrant used. In this case the ideal stoichiometric of the stronger acid was not equal to the real stoichiometric. The titration data range of two mixed weak acids used by Method A included such a deviation described. This method could not yield accurate and reproducible results of  $V_{eA}$  determination. But when the system of the acid mixtures which their  $\Delta pK_a$  was larger and the initial concentration ratios ratio was equal 1 or

nearby 1 (0.5), in the first buffer region of titration, the weaker acid did not ionize, the ideal stoichiometric of the stronger acid was nearly equal to the real stoichiometric itself. Gran's deviation was less effective to the raw data range chosen by Method A than the former one. The accurate results could be obtained from this method when the  $\Delta pK_a$  of the acid mixtures was larger ( $>2$  in this experiment). Although the  $\Delta pK_a$  of the acid mixtures was larger, the accurate results could not be obtained if the initial concentration ratios was much or less far from 1 (as in this experiment, the initial concentration ratios were 1 and 0.5). The reason might be that when the initial concentration ratios much differed from 1, in the final part of the first buffer region of the titration curve, the weaker acid ionized and reacted with the titrant. The stoichiometric deviation occurred and the accurate result could not be obtained. Another reason to explain why the initial concentration ratios ( $X = 1, 0.5$ ) of those acid mixtures in this experiment could yield the accurate results by using this method was that the stronger acid of those acid mixtures were benzoic acid and pivalic acid. The Gran plots of both acids gave the straight line in the region near the equivalent points when the equivalent volumes of benzoic acid were 2 and 4 ml (gave the initial concentration ratios of the acid mixtures  $X = 1$  and 0.5 respectively) and the region near the equivalent points when the equivalent volumes of pivalic acid were 2 ml (gave the initial concentration ratios of the acid mixtures  $X = 1$ ). Gran's deviation of these acid mixtures was so small; thus, Method A could be used to yield the accurate result of  $V_{eA}$  and  $V_{eB}$  determination in such situation.

2.2 Method B : For this method,  $V_{eA}$  could be determined by interpretation of titration data in the range of  $V_i$  to  $V_{fA}$  and  $V_{eB}$  could be determined in the same manner as  $V_{eA}$ . The raw data of acid mixture titrations would be interpreted in the range of  $V_i$  to  $V_{fB}$

where  $V_i$  = the volume of titrant which began to give maximum  $r^2$  in Gran plots of each single weak acids. (Compared these values between acid A and acid B, then the less value was selected.)

$V_{fA}$  = 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.

From table 119, it could be seen that the accurate and reproducible results could be obtained by this method of all the acid mixtures in this study (except the pralidoxime chloride - boric acid mixtures which would be discussed later) if the initial concentration ratios were not 15 and 0.1. The initial concentration ratio when X was equal 15 was called the upper limit initial concentration ratio and X was 0.1, the lower limit initial concentration ratio.

The reasons for describing that why this method was the appropriate method used for choosing the range of titration data were the same as those described in Method A.

For the acid mixtures which the initial concentration ratios was the upper limit initial concentration ratio, the accurate results could not be obtained by this method. In such condition the initial concentration of acid A and acid B was about 0.064 mol/litre (M). Potassium chloride was the neutral and inert electrolyte added to the titration solution for maintaining virtually constant ionic strength so that the activity coefficients and the liquid - junction potential did not vary during the course of titration (schwartz : 1989). The concentration of potassium chloride not only remained exactly constant throughout each titration, but should be at least two orders of magnitude larger than the concentration of the initial concentration of the acid being titrated (Barry, Meites and Campbell ; 1974). In such a system that the initial concentration of acid mixtures was about 0.064 M, potassium chloride used in this experiment (0.1 M ) might not be served to maintain the liquid junction potential of the cell used for pH measurement in potentiometric titration. Another reason was that the first buffer region of these acid mixtures was very low buffer region. The measurement of pH and volume of base were known to less precise in these regions (the details would be discussed in the next part). Furthermore, the change of ionic strength in the acid mixtures of ionized weak acid mixtures which initial concentration ratio was 15 were comparatively high, ionic strength changed from initial about 0.15 to 0.10 at the end of titration (approximately 50% change). The changes of ionic

strength that increasing arised for large value of X of those acid mixtures might cause appreciable deviation of activity coefficient determination equation propose by Davies,1964. A rough approximation of activity coefficient might be occured in that situation. From these reasons, the accurate results could not be obtained in such a system whether any one of all the methods was used for choosing the raw data range.

For the acid mixtures whose the initial concentration ratios was the lower limit initial concentration ratio, the accurate results could not be obtained by this method. In such condition the second buffer region of the titration curve was very low, comparing with the second buffer region of other acid mixtures which the initial concentration ratio was not the lower limit initial concentration ratio. The measurement of pH were known to be less precise in poorly buffered solutions than in strong ones; therefore, the data were least reliable in the regions where the curves were steeper. Glass electrode were ill - behaved in poorly buffered solutions, where the standard error of a measurement was more. Moreover, the pH values obtained in those regions might be drastically affected by traces of impurities (Kateman ; Smit and Meites ; 1983). The standard errors of a measured pH value also increased as the buffer capacity of the solution decreased (Smit, Meites and Kateman ; 1983). Furthermore, in the regions where the curve was steep, errors in measurements of volume of titrant ( $V_b$ ) did become significant, and might greatly outweigh those in measurements of pH (Kateman ; Smit and Meites ; 1983). Since the acid mixtures whose the initial concentration ratio was 0.1 ( $X = V_{eB}/V_{eA} = 2 \text{ ml}/ 20 \text{ ml}$ ), the increment of the titrant volumes used in the second

buffer region of the titration curve was 0.05 ml. Since the standard error of the automatic titrator was limited to constant values owing to the efficiency of the equipments, the relative error was large at small increments of titrant volume. As a consequence, two significant errors were achieved for the acid mixtures which their initial concentration ratios were about 0.1. Then, the accurate results could not be obtained by all the methods used.

For the pralidoxime chloride - boric acid mixture which the initial concentration ratios were about 5 and more than that, the accurate results could not be obtained by using Method B. Since it was known that when the acid had the high dissociation constants and high concentrations, it might cause appreciable deviation from linearity of the Gran plots due to nonquantitativeness of the titration reaction (Macca and Bombi ; 1989). Among acid mixtures studied, boric acid was the highest dissociation constant acid and when the high concentration was used (0.02 M for  $X = 5$  and 0.04 M for  $X = 10$ ), deviation from Gran plots linearity range was noticeably appeared in the titration data range chosen by Method B, leading to error in  $V_{eB}$  determination. Moreover, the difference between pKa value of this acid mixtures was 1.14 which was so small comparable to the value obtained from the other acid mixtures; hence, in the titration region comparing with the first region of single acid Gran plots of boric acid, pralidoxime chloride incompletely reacted with the titrant, as a result, the stoichiometric deviation of boric acid might become larger comparable to the other boric acid mixtures.

2.3 Method C : From this method, raw data of two-mixed weak acids titration would be chosen in the range of  $V_I$  to  $V_F$  which gave maximum F value. The pairs of weak acids mixtures which had no statistical difference at 95% confidence interval between the equivalent volumes obtained from multiple linear regression analysis of two-mixed weak acids titration and G plot of each single weak acids titration were the acid mixtures which their difference between pKa values of weak acids much more than 2 and their initial concentration ratios 1 or 0.5 .

For the other pairs of weak acids mixtures, this method could not yield accurate and reproducible results. It might be due to the number of data points chosen by Method C for  $V_{eA}$  and  $V_{eB}$  determination in the mixture of those acid mixtures were nearly equal to those chosen by Method A which differed from the other pairs of acids mixtures as shown in Table 119. In 1993, Chiewcharnwattana used this method for choosing titration data range of two mixed weak acids and determined the equivalent volumes of the individual weak acids by multiple linear regression analysis. It was found that the accurate and reproducible results could not be obtained when the difference between pKa value of acids were less than 2, the same results as this study. Therefore, the multiple regression analysis of titration data range chosen by this method could not yield accurate equivalent volumes of the individual weak acids if pKa values between each weak acids were less than 2, and the initial concentration ratios was not about 1 or 0.5.



### III. The effect of $\Delta pK_a$

From Table 119, it would be found that the equivalent volumes of individual acids obtained from the acid mixture titrations ( $\Delta pK_a > 0.9$ ) were statistical indifference at 95% confidence interval from the equivalent volumes obtained from Gran plots of each single weak acid titrations if the initial concentration ratios were not the upper and lower limit of the initial concentration ratios in this experiment. By using the appropriate method for choosing the raw data range, the equivalent volumes of the individual acids obtained from the acid mixtures could yield accurate results.

In 1996, Prachasitthasak used the Method B for choosing titration data range of two-mixed weak acids and determined the equivalent volumes of the individual weak acid by multiple linear regression analysis. It could be found that the accurate and reproducible results could not be obtained when the difference between  $pK_a$  value of acids were less than 0.8. The range of data used for analysis was in the range of high buffer capacity. The slopes of buffer region of these titration curves reflected the apparent minimum slope value. In this region of acids mixtures which their  $pK_a$  values were quite close, the measurement of pH value of combined glass electrode was not sensitive enough to the change of titrant volume and could not separate the effect of each weak acids on pH of the solutions. The complication often arised owing to the fact that the difference between  $pK_a$  values of two acids was too small to permit the methods used. Error would arise when the

change in pH of the solution was too small, comparing to the change of titrant volume; thus, the error in determination of the equivalent volumes of the individual acid of the acid mixtures titration was observed.

#### **IV. The effect of the ionic strength of solution during titration**

For the titration of the acid mixtures which the initial concentration ratios was 15 ( $X = V_{eB}/V_{eA} = 30\text{ml}/2\text{ml}$ ), the equivalent volume of the individual acid obtained was statistical difference from the equivalent volume obtaining from Gran plots of each single weak acid titration. Although  $\Delta pK_a$  of these acids were more than 0.9 and Method B was used for choosing titration data range, accurate and reproducible results could not be obtained. The reasons for these acid mixtures were already described. Potassium chloride was the inert electrolyte added to the titration solution for maintaining virtually constant ionic strength so that the activity coefficient and the liquid-junction potential did not vary during the course of titration (Schwartz : 1989). The titration had to be carried out in the presence of a swamping electrolyte that would prevent variations of the activity coefficients and the liquid-junction potential (Smit and Meites ; 1983). It was necessary that the concentration of potassium chloride had to remain constant throughout each titration with at least two orders of magnitude larger than the concentration of the initial concentration of the acid being titrated (Barry, Meites and Campbell ; 1974). Liquid junction potential was the function of the e.m.f. of the cell used for pH measurement which might be described by the following equation (Ingman, Johansson and Karlsson ; 1973).

$$E = E_0' + (RT/nF) \ln [H^+] + E_j'$$

$E_0'$  was a constant depending on the type of cell. The second term  $(RT/nF) \ln [H^+]$ , the “Nernst term”, was affected by the variation of the concentration of hydrogen ion  $[H^+]$ . The third term,  $E_j'$ , included the liquid junction potential and termed for the variation of the activity factors with changing acidity of the solution; thus, variation of liquid junction potential affected to pH measurement as described by this function.

Moreover, the error of pH measurements might be occurred by the liquid junction potential variation resulting from the difference in the composition of the standard buffer and the unknown solution (Skoog et al., 1990).

Since the potentiometric titrations were sometimes performed in concentrated ionic media; then, variation of the activity coefficients of the ions might be obtained and affected to the liquid junction potential of cell. As a result, the error in pH measurement was achieved.

#### **V. The effect of the initial concentration ratios (X) of the acid mixtures**

The effect of the variation of X was a more complex way ; since, it involved the relative precision of both  $V_{eA}$  and  $V_{eB}$ . Investigation

concerning how the accurate results in determining  $V_{eA}$  and  $V_{eB}$  by using multiple linear regression analysis with suitable titrations had been overwhelmingly influenced by the behaviors of the shapes of titration curves or the slopes, indeed. The effects of  $\Delta pK_a$  of the acid mixtures also involved in the shapes of the titration curves. Of course, a point of maximum slope was better defined, and the equivalent points could be located more precisely if the variations of slope were large corresponding to the titration mixtures which the difference between the  $pK_a$  value was large. A much more important consideration was that the raw data range being interpreted was in the region of the titration curve with different buffer capacity. From the experiment, even if the acid mixtures with the same  $\Delta pK_a$  but difference in the initial concentration ratios ( $X$ ), the shapes of the titration curves were different. The consideration was that the titration curves could be interpreted into two region : the first region was the buffer region which the stronger acid dominantly reacted with the titrant, the second region was the buffer region which the weaker acid noticeably reacted with the titrant.

Both regions were used to evaluate the equivalent volume of each single acid from the two weak acid mixtures. The more reliable raw data range used, the more accurate results could be obtained. The buffer regions affected by the initial concentration ratios could be classified into two types :

### 1. High buffer region

High buffer region was the buffer region which its slope was comparatively less than any other regions as obviously occurred in the previous study of Chiewcharnwatana, 1993 and Prachasitthisak, 1996. In those experiments, pKa values were quite close and the initial concentration ratio was nearly equal 1.

The values of these slopes reflected the “apparent minimum slope value”. In this region, the measurement of pH value of combined glass electrode was not sensitive enough to the change of titrant volume and could not separate the effect of each weak acids on pH of the solutions. The complication often arised owing to the fact that the difference between pKa values of two acids was too small to permit the methods used in this study. Error would arise when the change in pH of the solution was too small, comparing to the change of titrant volume; consequently, it would lead to the error in determination of the equivalent volumes of the individual acids of the acids mixtures titration. Moreover, for data that were confined to the well-buffered portion of a titration curve, the error in the volume of titrant was nearly insignificant in comparison with the error in pH; therefore, the error in pH was worth taking into account (Kateman, Smit and Meites; 1983). From the above reasons, the accurate results could not be obtained in the acid mixtures which high buffer region raw data was chosen.

## 2. Low buffer region

Low buffer region was the buffer region which its slope was comparatively more than any other regions as obviously occurred in the titration curve of the acid mixtures which their initial concentration ratios were 0.1 ( $X = V_{e_B}/V_{e_A} = 2\text{ml}/20\text{ml}$ ) and 15 ( $X = V_{e_B}/V_{e_A} = 30\text{ml}/2\text{ml}$ ) as described in the previous part of this investigation. In this region, pH measurements by glass electrode was known to be less precise than in well buffered one because equilibrium between the bulk solution titrated and the layer of solution at the surface of a membrane of electrode was achieved slowly (Kateman, Smit and Meites, 1983; Skoog et al., 1990). Therefore the data were least reliable in the regions where the curves were steeper. Glass electrode was also known to ill-behaved in poorly buffered solution and its response would usually be slow and subjected to interfering ions such as traces of impurities, carbonate in a neutralization titration (Akimoto, Hanakuma and Hozumi, 1987). Moreover, the standard error of a measurement in this region became greater. Not only the standard errors of a measured pH value increased as the buffer capacity of the solution decreased but also the standard errors in measurements of volume of titrant ( $V_b$ ) did become significant and might greatly outweigh those in measurements of pH (Kateman, Smit and Meites; 1983). From the above reasons and also from the example of the acid mixtures which their initial concentration ratios were about 0.1 or 15 as described previously, the accurate results could not be obtained from the acid mixtures which very low buffer region raw data were chosen.

Only the acid mixtures which their  $\Delta pK_a$  and their initial concentration ratios were in intermediate strength gave the accurate equivalent volumes of each weak acids by using appropriate method for choosing raw data range in intermediate buffer regions.

#### **VI. The effect of the standard errors of pH measurement and volume of base.**

Generally in the experimental work , three main types of errors might occurred (Haswell , Brereton , 1990) : gross , systematic and random errors.

The gross error was probably the most obvious and could be attributed to problems such as instrumental breakdown or severe reagent or sample contamination . In this experiment, this type of errors could be minimize by using the well checked instrument and less contaminate solvent and reagents; e.g., distilled water.

Systematic error occurred as a result of some bias in the procedure such as an incorrectly calibrated instrument or the use of wrong or inaccurate volumetric glassware. Thus, for minimizing this error in this experiment automatic titrator and pH-meter was recalibrated each time before titration.

The final type of error , random error, arised from uncontrolled conditions such as electronic noise in a pH meter and the nearly exact

volume of base dispensed from pump revolution of titrant automatic dispenser. As a consequence, experimental titrations always involved random errors of measurement of both the pH and the volume of base. The effects of these random errors on the theoretically attainable precisions of the equivalent volumes ( $V_{eA}$  and  $V_{eB}$ ) obtained by multiple linear regression model could be studied by using Eq.191.

This equation used to predict the theoretically precisions of the equivalent volumes ( $V_{eA}$  and  $V_{eB}$ ) obtained by multiple linear regression analysis. For any values of the dissociation constant of acid A and acid B ( $K_{aA}$ ,  $K_{aB}$ ), the initial concentration ratios ( $X$ ) of both acid A and acid B, ionization product constant of water ( $K_w$ ), concentration of titrant used (normality of sodium hydroxide) and initial volume of solution being titrated ( $V_0$ ), the coefficient variations of the equivalent volumes ( $\%C.V. V_{eA}, \%C.V. V_{eB}$ ) were shown in table 123 to 134. The coefficient variation ( $\% C.V.$ ) or the relative standard deviation (RSD) was used to express the random error of the pH measurement and volume of base. From Table 123 to 134, it could be clarify that  $\% C.V.$  of both  $V_{eA}$  and  $V_{eB}$  increased for smaller values of  $\Delta pK_a$ , in the same manner as those obtained from the experiment. Since it would be expected on the basis of traditional methods for interpreting titration curves. Another reason was that the presence of the weaker acid when  $\Delta pK_a$  became smaller had substantial effects on the pH values at and near the initial of the titration of the weaker acid, and also on the points in the region near the first equivalent volume (Betti, Papoff and Meites; 1986). From these reasons, the precision of the equivalent volume detection was poor when  $\Delta pK_a$  was small.

When regarding with the initial concentration ratios ( $X$ ), % C.V. of  $V_{eA}$  was also increased and % C.V. of  $V_{eB}$  was decreased when initial concentration ratios ( $X$ ) was increased. The reason might be that the propagation of random error could be reduced by a larger amount of substances (Macca, Bombi; 1989). When the initial concentration ratios ( $X = V_{eA} / V_{eB}$ ) was large, the amount of acid B was larger than acid A; thus, % C.V.  $V_{eB}$  was decreased and % C.V.  $V_{eA}$  was increased. On the other hands, % C.V. of  $V_{eA}$  decreased and % C.V. of  $V_{eB}$  increased when initial concentration ratios ( $X$ ) decreased. The reason when the initial concentration ratios was small might be the same as described earlier. This conclusion could also be explained by the influence of the behavior of the slope of titration curves as described in the previous section. When the initial concentration ratio ( $X$ ) was small, the second buffer region of the titration curve was very low and when the initial concentration ratio ( $X$ ) was large, the first buffer region of the titration curve was very low. In these regions, the relative standard error of pH measurement and volume of base did become significant. Such effect arised noticeably in % C.V. of  $V_{eB}$  determination which acid B was in the low buffer region ( $X$  was small) and in % C.V. of  $V_{eA}$  determination which acid A was in the low buffer region ( $X$  was large).

By using Eq.191, the theoretically precisions of the equivalent volumes ( $V_{eA}$  and  $V_{eB}$ ) which performed by difference data acquisition patterns, pH meters, titrant dispensers and other parameters could be

theoretically predicted. The comparison of the precisions obtained might be used to evaluate the accuracy and precision between the methodology adopted. In general, for a routine instrumental analytical method, an RSD or % C.V. of less than 2 % was usually acceptable (Has wall, Brereton;1990).



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