CHAPTER IV

RESULT AND DISCUSSION

The eight acidic compounds which their properties were weak acid with pKa value between 4-9 , could dissolve in 0.1 M KCl solution and had no precipitating during the titration, were studied. These compounds were benzoic acid , potassium biphthalate , *p*- nitrophenol , vanillin , pralidoxime chloride , lidocaine hydrochloride , salicylamide , and procaine hydrochloride. Their pKa values were shown in Table 2. The scope of this research was to study the binary mixtures of weak acids which had the difference in pKa values (ΔpKa) less than 2 . 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 shown in Table 3 .

The binary mixtures of weak acidic compounds in this study could be 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 (G plot) of the titration of each single acid titrations to determine whether their was a statistical difference between these results . The student t-test at 95 % confidence interval was employed . Glan's plot (G plot) could be used as the reference method , since it had been shown in the former study (Seksiri 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 titrtration of two neutral weak acids mixtures

The neutral weak acid mixtures in this study were

1.1 *p*-nitrophenol and vanillin mixture

1.2 vanillin and salicylamide mixture

1.3 *p*-nitrophenol and salicylamide mixture

1.1 *p*-nitrophenol and vanillin mixture

The dissociation constants of these compounds as determined by G plots were 9.74×10^{-8} (pKa = 7.01) for *p*-nitrophenol and 5.26×10^{-8} (pKa = 7.28) for vanillin . The difference between pKa values of these acids was 0.27 . From the titration of each weak acid solutions , the equivalence volumes could be determined from the titration curves and G plots as shown in Figure 9 , 10, 17 , and 18 . The titration curve of these acids mixture was shown in Figure 23 and the results of these titrations were summarized in Table 4 and 5 .

It could be seen that there were statistical differences between the equivalence volumes of the individual weak acids obtained from the titration of these weak acids mixture and the equivalence volumes of the corresponding compounds obtained from G plots of each single weak acid titrations . Thus , the modified equation (Eq.69) and all methods used for choosing the range of raw data in this study could not be used to determine accurate and reproducible equivalence volumes of the individual weak acids from these acids mixture titration.(see Table 34)

1.2 Vanillin and salicylamide mixture

The dissociation constants of these compounds as determined by G plots were 5.26×10^{-8} (pKa = 7.28) for vanillin and 7.94×10^{-9} (pKa = 8.10) for salicylamide . The difference between pKa values of these acids was 0.82 . From the titration of each weak acid solutions, the equivalence volumes could be determined from the titration curves and G plots as shown in Figure 10 , 13 , 18 , and 21 . The titration curve of these acids mixture was shown in Figure 24 and the results of these titration were summarized in Table 6 and 7 .

It could be seen that the equivalence volumes of the individual weak acids obtained from the titration of these acids mixture , when Method A , B , D , and E were used for choosing the range of raw data , were statistical indifferent from those obtained from G plots of each single weak acid titrations . But for choosing the range of raw data by method C , there were statistical differences between the equivalence volumes of each weak acids obtained from the acid mixture titration and those obtained from G plot of each single acid titrations. (see Table 34)

As considered to Table 37 , it would be seen that the number of data points chosen by Method A , B , D and E for equivalence volumes determining of both vanillin and salicylamide (Ve_A and Ve_B) were nearly equal when compared to the other pairs of weak acid mixtures. Whereas the numbers of data points chosen by Method C was different from those chosen by these method. Thus , if Ve_A and Ve_B obtained by Method D were accurate, $Ve_A$and Ve_B obtained by Method A , B and E would be accurate too since the ranges of titration data chosen by Method A , B , and E for Ve_A and Ve_B determining approached to those chosen by Method D.

1.3 *p*-nitrophenol and salicylamide mixture

The dissociation constants of these compounds as determined by G plots were 9.74×10^{-8} (pKa = 7.01) for *p*-nitrophenol and 7.94×10^{-9} (pKa = 8.10) for salicylamide . The difference between pKa values of these acids was 1.09 . From the titration of each weak acid solutions, the equivalence volumes could be determined from the titration curves and G plots as shown in Figure 9 , 13 , 17 , and 21 . The titration curve of these acids mixture was shown in Figure 25 and the results of these titrations were summarized in Table 8 and 9 .

The equivalence volumes of the individual acids obtained from the titration of these acids mixture , when Method D was used for choosing the range of raw data , were statistical indifferent from those obtained from G plots of each single weak acid titrations. For choosing the range of raw data by the Method A , B , C , or E , there were statistical differences between the equivalence volumes of each weak

acids obtained from the acid mixtures titration and those obtained from G plots of each single acid titrations . (see Table 34)

From the results of this group (the mixtures of two neutral weak acids), it could be concluded that Method D was the best method for choosing titration data range to determine equivalence volumes of each weak acids. The pairs of weak acid mixtures which their equivalence volumes could be determined by the multiple linear regression analysis and computer program, SPSS/PC$^+$ were

1. *p*-Nitrophenol and salicylamide mixture (ΔpKa = 1.09)

2. Vanillin and salicylamide mixture (ΔpKa = 082)

For the mixture of *p*-nitrophenol and vanillin (ΔpKa = 0.27), the equivalence volumes of the individual acids could not be determined accurately by all methods used for choosing the range of raw data in this study.

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

2.1 Lidocaine hydrochloride and Salicylamide mixture

The dissociation constants of these compounds as determined by G plots were 1.11×10^{-8} (pKa = 7.95) for lidocaine hydrochloride and 7.94×10^{-9} (pKa = 8.10) for salicylamide. The difference between pKa values of these acids was 0.15 . From the titration of each single weak acid solutions, the equivalence volumes could be determined from the titration curves and G plots as shown

in Figure 12 , 13 , 20 , and 21. The titration curves of these acids mixture was shown in Figure 26 and the results of these titrations were summarized in Table 10 and 11 .

It could be seen that the equivalence volumes of the individual weak acids obtained from the titration of this mixture were statistical different from those obtained from G plots of each single weak acid titrations. (see Table 34)

For this pair of weak acid mixture , the dissociation constants of these compounds were quite close (ΔpKa = 0.15). The measurement of pH value of the combined glass electrode was not sensitive enough to the changes of titrant volumes . All methods used for choosing the range of raw data in this study which would be applied into the modified equation (Eq.81) could not be used for determining accurate equivalence volumes of the individual weak acids from this binary acids mixture titration .

2.2 Pralidoxime chloride and salicylamide mixture

The dissociation constants of these compounds as determined by G plots were 1.15×10^{-8} (pKa = 7.94) for pralidoxime chloride and 7.94×10^{-9} (pKa = 8.10) for salicylamide . The difference between pKa values of these acids was 0.16 . From the titration of each weak acid solutions , the equivalence volumes could be determined from the titration curves and G plots as shown in Figure 11, 13 , 19 , and 21 . The titration curve of these acids mixture was shown in figure 27 and the results of these titrations were summarized in Table 12 and 13 .

The equivalence volumes of each weak acids obtained from the titration of these acids mixture were stastistical different from those

obtained by G plots of each single weak acid titrations. (see Table 34) All methods used for choosing the range of raw data in this study which would be applied into the modified equation (Eq.81) could not be used for determining accurate equivalence volumes of the individual weak acids from this binary acids mixture titration .

2.3 Vanillin and pralidoxime chloride mixture

The dissociation constants of these compounds as determined by G plots were 5.26×10^{-8} (pKa = 7.28) for vanillin and 1.15×10^{-8} (pKa = 7.94) for pralidoxime chloride . The difference between pKa values of these weak acids was 0.66 . From the titration of each single weak acid solutions, the equivalence volumes could be determined from the titration curves and G plots as shown in Figure 10, 11 , 18 ,and 19 . The titration curve of these acids mixture was shown in Figure 28 and the results of these titrations were summarized in Table 14 and 15 .

It could be seen that the equivalence volumes of the individual weak acids obtained from the titration of these acids mixture were statistical different from those obtained by G plots of each single weak acid titrations(see Table 34). All methods used for choosing the range of raw data in this study which would be applied into the modified equation (Eq.81) could not be used for determining accurate equivalence volumes of the individual weak acids from this binary acids mixture titration .

2.4 Vanillin and lidocaine hydrochloride mixture

The dissociation constants of these compounds as determined by G plots were 5.26×10^{-8} (pKa = 7.28) for vanillin and 1.11×10^{-8}

(pKa = 7.95) for lidocaine hydrochloride . The difference between pKa values of these compounds was 0.67 . From the titration of each weak acid solutions, the equivalence volumes could be determined from the titration curves and G plots as shown in Figure 10 , 12 , 18 , and 20 . The titration curve of these acids mixture was shown in Figure 29 and the results of these titrations were summarized in Table 16 and 17 .

It could be seen that there were statistical differences between the equivalence volumes obtained from the titration of these acids mixture and those obtained from G plots of each single acid titrations (see Table 34). All methods used for choosing the range of raw data in this study which would be applied into the modified equation (Eq.81) could not be used for determining accurate equivalence volumes of the individual weak acids from this binary acids mixture titration . As considered to Table 16 , it would be seen that the equivalence volumes of vanillin obtained from Method A and B of these acids mixture titration in the first experiment of five replicates were fluctuated from the others. The Q-test was used as a statistical test to decide whether to retain or reject these results (Skoog et al., 1990). From this statistical test , these results should be rejected at 96 % confidence level.

2.5 *p*-Nitrophenol and pralidoxime chloride mixture

The dissociation constants of these compounds as determined by G plots were 9.75×10^{-8} (pKa = 7.01) for *p*-nitrophenol and 1.15×10^{-8} (pKa = 7.94) for pralidoxime chloride . The difference between pKa values of these weak acids was 0.93 . From the titration of each single weak acid solutions , the equivalence volumes could be determined from the titration curves and G plots as shown in Figure 9,

11 , 17 , and 19 . The titration curve of these acids mixture was shown in Figure 30 and the results of these titrations were summarized in Table 18 and 19 .

It could be seen that there were statistical indifferences between the equivalence volumes of the individual acids obtained from the titration of these acids mixture and the equivalence volumes of the corresponding compounds obtained from G plots of each single weak acid titrations if Method D was used for choosing the range of raw data. By the other methods of choosing titration data range , the equivalence volumes of the individual acids obtained from the titration of the acid mixture were statistical different from those obtained from G plots of each single weak acid titrations. (see Table 34)

2.6 *p*-Nitrophenol and lidocaine hydrochloride mixture

The dissociation constants of these compounds as determined by G plots were 9.75×10^{-8} (pKa = 7.01) for *p*-nitrophenol and 1.11×10^{-8} (pKa = 7.95) for lidocaine hydrochloride . The difference between pKa values of these weak acids was 0.94 . From the titration of each single weak acid solutions , the equivalence volumes could be determined from the titration curves and G plots as shown in Figure 9 , 12 , 17 , and 20 . The tiration curve of these acids mixture was shown in Figure 31 and the results of these titrations were summarized in Table 20 and 21 .

It could be seen that there were statistical indifferences between the equivalence volumes of the individual acids obtained from the titration of these weak acids mixture and the equivalence volumes of the corresponding compounds obtained from G plots of each single weak

acid titrations if the method used for choosing the range of raw data was Method D. By the other methods , the equivalence volumes of the individual weak acids obtained from the titration the acid mixture were statistical different from those obtained from G plots of each single weak acid titrations (see Table 34) . The results of these acids mixture titrations were the same manner as the results of the titration of p-nitrophenol and pralidoxime chloride mixture .

2.7 Benzoic acid and potassium biphthalate mixture

The dissociation constants of these compounds as determined by G plots were 8.71×10^{-5} (pKa = 4.06) for benzoic acid and 9.88×10^{-6} (pKa = 5.01) for potassium biphthalate . The difference between their pKa values was 0.95 . From the titration of each single weak acid solutions , the equivalence volumes could be determined from the titration curves and G plots as shown in Figure 7 , 8 , 15 , and 16. The titration curve of these acids mixture was shown in Figure 32 and the results of these titrations were summarized in Table 22 and 23 .

The equivalence volumes of the individual acids obtained from the titration of these binary acids mixture by all methods used in this study were statistical different at 95% confidence interval from those obtained from G plots of each single weak acid titration.

2.8 Salicylamide and procaine hydrochloride mixture

The dissociation constants of these compounds as determined by G plots were 7.94×10^{-9} (pKa = 8.10) for salicylamide and 8.51×10^{-10} (pKa = 9.07) for procaine hydrochloride . The difference between pKa values of these weak acids was 0.97 . From the titration

of each weak acid solutions ,the equivalence volumes could be determined from the titration curves and G plots as shown in Figure 13 , 14, 21 , and 22. The titration curve of these weak acids mixture was shown in Figure 33 and the results of these titration were summarized in Table 24 and 25 .

The equivalence volumes of the individual acids obtained from the titration of these binary acids mixture were statistical indifferent from those obtained from G plot of each single single weak acids titration if the methods used for choosing the range of raw data were Method B and D . By Method A , C and E , the equivalence volumes of the individual weak acids obtained from the titration of these acids mixture were statistical different at 95% confidence interval from those obtained from G plots of each single weak acid titration. It would be seen that Method B could yield accurate and reproducible results in this pair of weak acids mixture whereas in the other pairs of acid mixtures titrations , accurate and reproducible results could not be obtained by this method. The reason was about statistics which would be discussed later (see page 68).

2.9 Vanillin and procaine hydrochloride mixture

The dissociation constants of these compounds as determined by G plots were 5.26×10^{-8} (pKa = 7.279) for vanillin and 8.51×10^{-10} (pKa = 9.07) for procaine hydrochloride . The difference between pKa values of these weak acids was 1.79 . From the titration of each weak acid solutions , the equivalence volumes could be determined from the titration curves and G plots as shown in Figure 10 , 14 , 18 , and 22 . The titration curve of these weak acids mixture was shown in Figure 34 and the results of these titrations were summarized in Table 26 and 27 .

It could be seen that , there were no statistical differences between the equivalence volumes of the individual weak acids obtained from the titration of these binary acids mixture and the equivalent volumes obtained from G plot of each single acid titrations if method used for choosing the range of raw data was Method D.

From the results of this group of acid mixtures (the mixtures between neutral and ionized weak acids) , it was concluded that if ΔpKa of the acids mixture was not below 0.8 , the equivalence volumes of the individual weak acids could be determined accurately by the modified multiple linear equation (Eq. 81) and Method D was the best method for choosing the titration data ranges applied to analysis (see Table 34). The pairs of weak acid mixtures which their equivalence volumes could be determined accurately were as followed .

1. *p*-Nitrophenol and pralidoxime chloride mixture. (ΔpKa = 0.93)
2. *p*-Nitrophenol and lidocaine hydrochloride mixture. (ΔpKa= 0.94)
3. Salicylamide and procaine hydrochloride mixture. (ΔpKa = 0.95)
4. Vanillin and procaine hydrochloride mixture. (ΔpKa = 1.79)

The pairs of acids mixtures which their equivalence volumes could not be determined by the multiple linear regression analysis though Method D was used for choosing the range of raw data , were as followed.

1. Lidocaine hydrochloride and salicylamide mixture. (ΔpKa = 0.15)
2. Pralidoxime chloride and salicylamide mixture. (ΔpKa = 0.16)
3. Vanillin and pralidoxime chloride mixture. (ΔpKa = 0.66)
4. Vanillin and lidocaine hydrochloride mixture. (ΔpKa = 0.67)
5. Benzoic acid and potassium biphthalate mixture. (ΔpKa = 0.95)

III The titration of two ionized weak acids mixtures

The ionized weak acids mixtures in this study were

3.1 Pralidoxime chloride and lidocaine hydrochloride mixture.

3.2 Lidocaine hydrochloride and procaine hydrochloride mixture.

3.3 Pralidoxime chloride and procaine hydrochloride mixture.

3.1 Pralidoxime chloride and lidocaine hydrochloride mixture.

The dissociation constants of these compounds as determined by G plots were 1.15×10^{-8} (pKa = 7.94) for pralidoxime chloride and 1.11×10^{-8} (pKa = 7.95) for lidocaine hydrochloride. The difference between pKa values of these acids was 0.01 . From the titration of each weak acid solutions , the equivalence volumes could be determined from the titration curves and G plots as shown in Figure 11, 12 , 19 , and 20 . The titration curve of these acids mixture was shown in Figure 35 and the results of these titrations were summarized in Table 28 and 29 .

From Table 28, 29 and 34, it could be seen that the equivalence volumes of the individual weak acids obtained from the titration of these acids mixture, were statistical different from those obtained from G plots of each single weak acid titrations. All methods used for choosing the range of raw data could not yield accurate results . The equivalence volumes of pralidoxime chloride (Ve_A) could not be determined from titrations of this pair of weak acids since the results obtained from SPSS/PC$^+$ program showed that variable x_1 was not in the equation. From Table 29 , it could be seen that the equivalence volumes of lidocaine hydrochloride (Ve_B) obtained from the mixed-weak acid titrations were nearly equal to the sum of these two acids since their pKa

values were quite close . So titrations of this pair of weak acid mixture approached to single acid titrations. Table 29 also showed G plot of acid mixture titrations. It would be seen that equivalence volumes obtained from G plot of this acid mixture titration were nearly equal to the sum of those obtained from G plot of each single acid titrations.

For this pair of acids mixture , the difference between pKa values of each weak acids was too small (ΔpKa = 0.015) so that they seem to be the same values. This situation made the variables x_1 and x_2 in the multiple linear equation (Eq.91) nearly equal. The multiple linear equation for this pair of acids mixture could be written in the form of simple linear equation , as followed

$$Y = a_1x_1 + a_2x_2$$

when $x_1 \approx x_2 \approx x$

$$Y = (a_1 + a_2)\,x$$

The computer program , SPSS/PC$^+$ showed that variable x_1 was not in the equation so the partial regression coefficient a_1 could not be obtained. Only variable x_2 was in the equation. It meant that only variable x_2 was influential on variable y in this multiple linear equation so only a_2 could be determined. From the value of a_2 , the equivalence volumes of acid B would be obtained. These equivalence volumes were nearly equal to the sum of these two acids.

3.2 Lidocaine hydrochloride and procaine hydrochloride mixture.

The dissociation constants of these compounds as determined by G plots were 1.11×10^{-8} (pKa = 7.95) for lidocaine hydrochloride and 8.51×10^{-10} (pKa = 9.07) for procaine hydrochloride . The difference between pKa values of these acids was 1.12 . From the titration of each weak acid solutions, the equivalence volumes could be determined from the titration curves and G plots as shown in Figure 12 , 14 , 20 and 22. The titration curve of these acids mixture was shown in Figure 36 and the results of these titrations were summarized in Table 30 and 31.

It could be seen that the equivalence volumes of the individual acids obtained from the titrations of these acids mixture when Method D was used for choosing the range of raw data , were statistical indifferent from those obtained from G plots of each single weak acid titrations. For choosing the range of raw data by the other methods , there were statistical differences between the equivalence volumes of each weak acids obtained from the acids mixture titration and the equivalence volumes obtained from G plots of each single weak acid titrations (see Table 34).

3.3 Pralidoxime chloride and procaine hydrochloride mixture

The dissociation constants of these compounds as determined by G plots were 1.15×10^{-8} (pKa = 7.94) for pralidoxime chloride and 8.51×10^{-10} (pKa = 9.07) for procaine hydrochloride . The difference between pKa values of these acids was 1.13 . From the titrations of each weak acid solutions , the equivalence volumes could be determined from the titration curves and G plots as shown in Figure 11 ,14 , 19 and 22. The titration curve of these acids mixture was shown in

Figure 37 and the results of these titrations were summarized in Table 32 and 33.

It could be seen that the equivalence volumes of the individual acids obtained from the titration of these acids mixture, when Method D was used for choosing the range of raw data , were statistical indifferent from those obtained from G plots of each single weak acid titrations. For choosing the range of raw data by Method A , B , C or E , there were statistical differences between the equivalence volumes of each weak acids obtained from these acids mixture titration and the equivalence volumes obtained from G plots of each single weak acid titrations (see Table 34).

From the results of this group (the mixtures of two-ionized weak acids), it was concluded that accurate equivalence volumes of the individual weak acids could be determined by the multiple linear equation (Eq.91) and the range of raw data applied to analysis should be chosen only by Method D. Thus, the multiple linear regression analysis and the computer program, SPSS/PC$^+$ could be used for determining the equivalence volumes of the individual acids from the binary weak acids mixtures such as lidocaine hydrochloride and procaine hydrochloride mixture and pralidoxime chloride and procaine hydrochloride mixture which their ΔpKa values were about 1.1 .

For the mixture of pralidoxime chloride and procaine hydrochloride , the multiple linear regression analysis and computer program , SPSS/PC$^+$ could not be used for determination of equivalence volumes of the individual acids from these acids mixture titration since

their pKa values were quite close so they were seem to be single acid titration.

From the results of these experiments (Table 34), it would be concluded that there were many factors which affected to the accuracy and precision of the equivalence volumes obtained from the titration of binary acids mixtures such as ; the titration data range applied for analysis , the differences between pKa values of weak acids , and the ionic strength of solution during the course of titration.

Factors affecting the accuracy and precision of the equivalence volumes of the individual weak acids obtained from the titration of binary acids mixtures which their ΔpKa were less than two

I. The effect of titration data range applied for analysis

From Table 34 , 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 equivalence volumes of the individual acids obtained. The appropriate method used for choosing titration data range was Method D. For this method, accurate and reproducible results would be obtained if the difference between pKa values of weak acids was more than 0.8 . For choosing the ranges of titration data by Method A , B , C or E , then applied these titration data ranges to the multiple linear equation , accurate and reproducible results could not be obtained though ΔpKa values of the acid mixtures were more than 0.8 . The results obtained from each methods could be discussed as followed.

1. Method A : For 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 equivalence volumes obtained from multiple linear regression analysis of two-mixed weak acids titration and G plot of each single weak acid titrations were vanillin and salicylamide mixture (ΔpKa = 0.82). Whereas the other pairs of weak acid mixtures , this method could not yield accurate and reproducible results. It might be due to the number of data points chosen by Method A for Ve_A and Ve_B determining in the mixture of vanillin and salicylamide were nearly equal to those chosen by Method D (the appropriate method) which differred from the other pairs of acids mixtures as shown in Table 37. In 1993, Supawadee Chiewcharnwattana used this method for choosing titration data range of two-mixed weak acids and determined the equivalence volumes of the individual weak acids by multiple linear regression analysis. It could be found that accurate and reproducible results could not be obtained when the difference between pKa value of acids were less than 2 . For this study , the results obtained were followed the conclusion of a previous study. Only a pair of weak acid mixture such as vanillin and salicylamide mixture which accurate results could be obtained. So the multiple regression analysis of titration data range chosen by this method could not yield accurate equivalence volumes of the individual weak acids if pKa values between each weak acids were less than 2.

2. Method B : For this method , 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}) , then Ve_A and Ve_B 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 differences between the equivalence volumes obtained from multiple regression analysis of two-mixed weak acids titration and G plots of each single acid titrations were

a. vanillin and salicylamide mixture ($\Delta pKa = 0.82$)

b. salicylamide and procaine HCl mixture ($\Delta pKa = 0.97$)

As considered to the other pairs of acid mixtures , this method could not yield accurate and reproducible results of Ve_A determining when compared to those obtained by Method D. 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 equivalence points of acid A (stronger acids). Thus, errors in Ve_A determining might be happen. Two reasons would be described , as followed.

2.1 The multiple linear equation used in this study was derived on the basic of mass balance , charge balance and equilibrium equation which was the same manner as the derivation of the equations in Gran's mathod 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) : positive deviation before the equivalence point and negative deviation in the initial part. Thus , the titration data range of two-mixed weak acids used for determination of Ve_A and Ve_B should be within the linearity range of Gran plots of each single acid titrations.

2.2 pH measurement by glass electrode was known to be less precise in poorly buffered solution such as in the region of inflection point of titration curve because equilibrium between the 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). The measurement should not be taken close to the equivalence point where the electrode response would usually be slow and subjected to interfering ions such as carbonate in a neutralization titration , due to the low level of the measuring ion (Akimoto, Hanakuma and Hozumi, 1987).

For vanillin and salicylamide mixture , the titration data range chosen by this method would be interpreted and yielded accurate and reproducible results. The reason was the same as described in Method A. The numbers of data point chosen by Method D and Method B for Ve_A and Ve_B determining of this pair of acid mixture were nearly equal.

For salicylamide and procaine hydrochloride mixture , reporting of analytical data such as the significant figures and rounding of data were influential on t-calculated value and the decision that whether there was a statistical difference between these results. As considered to Table 24 , the equivalence volume of salicylamide obtained by Method B was $2.39 \pm 1.45 \times 10^{-2}$ ml which was statistical indifferent from that obtained from G plot ($2.40 \pm 4.83 \times 10^{-3}$ ml) of single acid titration at 95% confidence interval (t-calculated value = 1.46 , t-critical value = 1.86). t-calculated value of the result was less than t-critical value so the equivalence volume of salicylamide obtained from the titration of these acids mixture (by Method B) was statistical indifferent from the equivalence volume obtained from G plot of single acid titration. But , if

data was reported as four significant figures , the equivalence volume of salicylamide obtained from this pair of acids mixture titration (by Method B) was $2.389 \pm 1.451 \times 10^{-2}$ ml and the equivalence volume of salicylamide obtained from G plot of single acid titration was $2.402 \pm 4.834 \times 10^{-3}$ ml . t-calculated value was 1.901 which was more than t-critical value . It would be seen that if data was reported as four significant figures , there was a statistical difference between the equivalence volume of salicylamide obtained from Method B of these acid mixture titration. The sensitivity of the instruments used in this study such as an automatic titrator and pH meter affected to the significant figures of data reported. Automatic titrator could delivered the volumes of titrant with three significant figures and pH meter could measure pH value with four significant figures so the experimental data was reported as three significant figures and in order to avoid rounding error it was important to postpone rounding until the calculation was completed. In this case , the reliability of the results would be increased by increasing the sensitivity of instruments and the quantity of the sample analysed.

For the equivalence volume determination of procaine HCl , the titration data range chosen by this method was the same as the range chosen by Method D so the result obtained by this method was as same as the result obtained by Method D.

3. Method C : For this method , 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 equal to $N_1 + N_2$ where N_1 and N_2 were the numbers of raw data obtained from the linearity range of G plot of each single

acid titrations which were used for determining the equivalence volumes and dissociation constants of acid A and acid B respectively.

As considered to Table 34 , it would be seen that the results obtained from this method of all pairs of acid mixture titrations were statistical different from those obtained from G plot of each single acid titrations. It might be due to the number of data points chosen by this method represented approximately 50% or less of the titration curve before equivalence point whereas the other methods used in this study represented more than 70% of the titration curve before equivalence point . The numbers of data points chosen by many methods used in this study were shown in Table 36 . In 1975 , Stanley and David studied the effects on titration error and end-point uncertainty which arised from measurement precision and the location on the titration curve of points used in making Gran plots. They concluded that data points used in Gran plot should represent as large a region of the titration curve as possible (approximately 60% or more in their study). The higher precision of the end point would be obtained when the larger numbers of data point were taken within the range closer to the equivalence point. (Akimoto et al., 1987)

4. Method D : For this method , Ve_A could be determined by interpretation of titration data in the range of V_I to V_{FA} and Ve_B could be determined in the same manner as Ve_A. 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 G 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 34 , it could be seen that accurate and reproducible results could be obtained by this method if the difference between pKa value of each weak acids were more than 0.8 (in this study). 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 B.

The pairs of weak acids mixtures which accurate and reproducible results could be obtained by this method , were as followed.

1. Vanillin and salicylamide mixture. (ΔpKa = 0.82)
2. *p*-Nitrophenol and pralidoxime chloride mixture. (ΔpKa = 0.93)
3. *p*-Nitrophenol and lidocaine HCl mixture. (ΔpKa = 0.94)
4. Salicylamide and procaine HCl mixture. (ΔpKa = 0.97)
5. *p*-Nitrophenol and salicylamide mixture. (ΔpKa = 1.09)
6. Lidocaine HCl and procaine HCl mixture. (ΔpKa = 1.12)
7. Pralidoxime chloride and procaine HCl mixture. (ΔpKa = 1.13)
8. Vanillin and procaine HCl mixture. (ΔpKa = 1.79)

The pairs of weak acid mixture which the equivalence volumes of the individual acids could not be determined by this method were as followed.

1. Pralidoxime chloride and lidocaine HCl mixture. (pKa = 0.01)
2. Lidocaine HCl and salicylamide mixture. (ΔpKa = 0.15)
3. Pralidoxime chloride and salicylamide mixture. (ΔpKa = 0.16)
4. *p*-Nitrophenol and vanillin mixture. (ΔpKa = 0.27)
5. Vanillin and pralidoxime chloride mixture. (ΔpKa = 0.66)
6. Vanillin and lidocaine HCl mixture. (ΔpKa = 0.67)
7. Benzoic acid and potassium biphthalate mixture. (ΔpKa = 0.95)

It could be seen that this method could not be used to determine accurate equivalence volumes of each weak acids from the acid mixture titrations if their pKa values were quite close (ΔpKa was less than 0.8 for this study). For benzoic acid and potassium biphthalate mixture titration, the equivalence volumes of the individual acids could not be determined by this method since the ionic strength of the solution were changed significantly during the course of titration. The reason would be described in the topic of changes of ionic strength during titration.

5. Method E : For this method , the ranges of data points interpreted were the same as Method D , but

- For Ve_A determining , the equivalence volume of the weaker acid (Ve_B) obtained from Method D was substituted into the multiple linear equation then it would be rearranged to simple linear equation and Ve_A could be determined.

- For Ve_B determining , the equivalence volume of the stronger acid (Ve_A) obtained from Method D was substituted into the multiple linear equation then Ve_B could be determined.

From Table 34 , it could be concluded that this method could not be used for determination of the equivalence volumes of the individual acids from two-mixed weak acids titrations. The reason might be due to the equivalence volumes (Ve_A or Ve_B) obtained from Method D , which was substituted into the multiple linear equation was not the actual true value . So there was a little error occured from substitution of this estimate true value into the multiple linear equation which might cause Ve_A or Ve_B obtained by this method statistical different from those obtained from G plot of each single acid titrations.

For the mixture of vanillin and salicylamide, this method could yield accurate and reproducible results. It might be due to Ve_A and Ve_B obtained by Method D were equal to the actual true value so there was no error occured in substitution of these values into the equation.

II <u>The effect of ΔpKa</u>

From Table 34 , it would be found that the equivalence volumes of the individual acids obtained from the acids mixture titrations which their ΔpKa values were more than 0.8, were statistical indifferent at 95 % confidence interval from the equivalence volumes obtained from G plots of each single weak acid titrations. The methods used in this study could not be used for determining the equivalence volumes of the acids mixtures which their ΔpKa were less than 0.8 , such as ; the mixture between *p*-nitrophenol and vanillin , the mixture between salicylamide and lidocaine HCl , the mixture between salicylamide

and pralidoxime chloride , the mixture between vanillin and pralidoxime chloride , the mixture between vanillin and lidocaine HCl , and the mixture between pralidoxime chloride and lidocaine HCl . For these acids mixtures , it could be seen that the range of data used for analysis was in the range of high buffer capacity as shown in Fig.23 , 26 , 27 , 28 , 29 , and 35 . The slopes of buffer region of these titration curves were less than those of the titration curves of the other pairs of weak acids mixtures which their ΔpKa values were more than 0.8 as shown in Table 35 . The values of these slopes reflected the " apparent minimum slope value ". In this region of acids mixtures which their pKa 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 solution. 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 , compared to the change of titrant volume and it would lead to the error in determination of the equivalence volumes of the individual acids of the acid mixture titration . Moreover , it had been shown in the former study that when the dissociation constants of the two acids were quite close , the range of data appiled into the modified equation (Eq.69) would reflect high buffer capacity and the measurement of pH value of the combined glass electrode used for this region was not sesitive enough to the change of titrant volumes (Supawadee , 1993).

For the mixture of pralidoxime chloride and lidocaine HCl , their pKa values were nearly equal (ΔpKa = 0.015). Thus , the variable x_1

and x_2 in the multiple linear equation of these acids mixture seemed to be the same value and could not be distinguished by the computer program , SPSS/PC$^+$. The equivalence volumes of the weaker acid obtained from these acids mixture titration were nearly equal to the sum of two acids.

III. The effect of the ionic strength of solution during titration

For the titration of benzoic acid and potassium biphthalate mixture , the equivalence volumes of the individual acids obtained were statistical different from the equivalent volumes obtained from G plots of each single weak acid titrations. Although ΔpKa of these acids were more than 0.8 (ΔpKa = 0.95) and Method D was used for choosing titration data range , accurate and reproducible results could not be obtained . For these acids mixture titration , the ionic strength of the solution would be increased during the course of titration since there were many ions occured in the solution especially a doubly charge ion such as phthalate ion which would be increased during the titration . The activity coefficients for singly charged species were less affected by changed in ionic strength than were the coefficients for ion with multiple charges as shown in Figure 38 (Pecsok , et al., 1976 ; Skoog et al., 1990). The variation of the activity coefficient of ions affected to the liquid junction potential of the cell used for pH measurement in potentiometric titration.

The e.m.f. of the cell used for pH measurement might be described by an equation consisting of three terms.

$$E = E'_0 + (RT/nF)\ln[H^+] + E'_j \qquad \text{Eq. 94}$$

E'_0 was a constant depending only on the type of cell . The second term could be called the " Nernst term " and accounted for the variation of E with the concentration of hydrogen ion $[H^+]$. The third quantity E'_j included the liquid junction potential and termed for the activity factors with changing acidity of the solution .

Nevertheless , the error of pH measurements might be occured by the liquid junction potential variation resulting from the differences in the composition of the standard buffer and the unknown solution (Skoog et al., 1990). For the mixture of benzoic acid and potassium biphthalate, the difference between the activity coefficient of hydrogen ion in the analyte solution and in the standard buffer was more than this difference of the other pairs of acids mixtures . If the activity is unity, the response was Nernstian. In solution containing high solute concentrations, the activity of the water in equilibrium with the glass membrane was less than unity. For the titraton of the mixture of benzoic acid and potassium biphthalate, the activity coefficient of hydrogen ion was less than unity . Thus , it might lead to error in the measurements of pH by the glass electrode (Christian , 1986 ; Braun , 1987).

The variation of the activity coefficients of the ions affected to the liquid junction potential of the cell used for pH measurement in potentiometric titration. The error of pH measurement might be occured by the liquid junction potential variation .